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TNT Degradation by Natural Microbial Assemblages at Frontal Boundaries Between Water Masses in Coastal Ecosystems (ER-2124)

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14. ABSTRACT

This limited scope, three year SERDP project involves determining the primary biogeochemical factors that control energetics metabolism by natural microbial assemblages in coastal systems. By correlating standard water quality measurements with degradation rates, we can predict turnover times and ecosystem capacity for energetics released into hydrodynamically similar, UXO-impacted ecosystems where access to site samples may be limited. During samplings in the Florida Keys and Lower Outer Banks, NC, USA, we found that mixing experiments between coastal end members (mangrove lagoon or Cypress bog water, respectively) and open ocean seawater resulted in more rapid rates of bacterial growth and aromatic contaminant mineralization (i.e., TNT, RDX, HMX and phenanthrene) than would have been predicted by interpolation of unmixed end members. Energetics and PAH mineralization rates in areas adjacent to Key West DoD sites suggest that contaminants in surface runoff from shoreside areas should be rapidly metabolized (i.e., hours to weeks) in the adjacent seawater and sediment. Ecosystem capacities for contaminant biodegradation were also determined for three North Carolina coastal estuarine systems: Newport River Estuary, New River Estuary and Bogue Sound. The analyses predicted how much energetics or phenanthrene could be released into the headwaters or DoD site of each system and be attenuated before exiting the estuary into adjacent ecosystems. Taken together, this work supports a site conceptual model where PAHs and energetics would be rapidly biodegraded by natural microbial assemblages were they to migrate from mangrove- or Cypress-dominated coastal systems to adjacent waterways in both tropical and temperate ecosystems, respectively. The finding that energetics and PAHs are rapidly biodegraded in nature is counter to the long standing tenets of environmental bioremediation but is well supported by the emerging scientific understanding that organic compounds are not inherently refractory based solely on their chemical structure. This emerging understanding of lack of chemical structure based recalcitrance has dramatic implications for the field of bioremediation and site cleanup.

15. SUBJECT TERMS

Bacteria	Corpus Christi	Energetics	Key West	North Carolina	Salt wedge
Bacterial production	Cypress bog	Estuaries	Mangrove	PAHs	2,4,6-trinitrotoluene
Biodegradation	DOC	Frontal boundary	Marine	Phenanthrene	
Coastal	Ecosystem capacity	HMX	Mineralization	RDX	

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LIST OF ACRONYMS

AUV: Autonomous Underwater Vehicle
CDOM: Colored Dissolved Organic Matter
CO_2: Carbon Dioxide
CTD: Conductivity-Temperature-Depth water sampling device
CuO: Cupric Oxide
d^{-1}: Per Day
DAT: 2,4-Diaminotoluene
DNT: 2,4-Dinitrotoluene
DO: Dissolved Oxygen
DOC: Dissolved Organic Carbon
DoD: Department of Defense
DOM: Dissolved Organic Matter
EEM: Excitation-Emission Matrix
FID: Flame Ionization Detection
GC/MS: Gas Chromatography/Mass Spectrometry
H_2SO_4: Sulfuric Acid
HMX: Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
kg^{-1}: Per Kilogram
L^{-1}: Per Liter
mCi: Millicurie
mL: Milliliter
MNA: Monitored Natural Attenuation
MRP: Munitions Response Program
NAVFAC: Naval Facilities Engineering Command
NaOH: Sodium Hydroxide
NFA: No Further Action
NG: Nitroglycerine
nm: Nanometers
NRL: Naval Research Laboratory
OCONUS: Outside the Continental United States
OM: Organic Matter
PAH: Polycyclic Aromatic Hydrocarbons
PARAFAC: Parallel Factor Analyses
POC: Particulate Organic Carbon
PSU: Practical Salinity Units
QSE: Quinine Sulfate Equivalents
RDX: 1,3,5-Trinitroperhydro-1,3,5-triazine
RPM: Remedial Program Manager
SUVA: Specific Ultraviolet Absorption
SERDP: Strategic Environmental Research and Development Program
TCA: Trichloroacetic Acid
TBD: To Be Determined
TNT: 2,4,6,-Trinitrotoluene
µg: Micrograms
UL-: Uniformly Labeled
USN: United States Navy
UUV: Unmanned Underwater Vehicle
UXO: Unexploded Ordnance

KEYWORDS

bacteria
bacterial production
biodegradation
coastal
Corpus Christi
Cypress bog
DOC
ecosystem capacity
energetics
estuaries
frontal boundary
HMX
Key West
mangrove
marine
mineralization
North Carolina
PAHs
phenanthrene
RDX
salt wedge
2,4,6-trinitrotoluene

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ABSTRACT

This limited scope, three year SERDP project involves determining the primary biogeochemical factors that control energetics metabolism by natural microbial assemblages in coastal systems. By correlating standard water quality measurements with degradation rates, we can predict turnover times and ecosystem capacity for energetics released into hydrodynamically similar, UXO-impacted ecosystems where access to site samples may be limited. During samplings in the Florida Keys and Lower Outer Banks, NC, USA, we found that mixing experiments between coastal end members (mangrove lagoon or Cypress bog water, respectively) and open ocean seawater resulted in more rapid rates of bacterial growth and aromatic contaminant mineralization (*i.e.,* TNT, RDX, HMX and phenanthrene) than would have been predicted by interpolation of unmixed end members. Energetics and PAH mineralization rates in areas adjacent to Key West DoD sites suggest that contaminants in surface runoff from shoreside areas should be rapidly metabolized (*i.e.,* hours to weeks) in the adjacent seawater and sediment. Ecosystem capacities for contaminant biodegradation were also determined for three North Carolina coastal estuarine systems: Newport River Estuary, New River Estuary and Bogue Sound. The analyses predicted how much energetics or phenanthrene could be released into the headwaters or DoD site of each system and be attenuated before exiting the estuary into adjacent ecosystems. Taken together, this work supports a site conceptual model where PAHs and energetics would be rapidly biodegraded by natural microbial assemblages were they to migrate from mangrove- or Cypress-dominated coastal systems to adjacent waterways in both tropical and temperate ecosystems, respectively. The finding that energetics and PAHs are rapidly biodegraded in nature is counter to the long standing tenets of environmental bioremediation but is well supported by the emerging scientific understanding that organic compounds are not inherently refractory based solely on their chemical structure. This emerging understanding of lack of chemical structure based recalcitrance has dramatic implications for the field of bioremediation and site cleanup.

OBJECTIVE

This work involves determining the primary biogeochemical factors that control TNT metabolism by natural microbial assemblages in coastal systems. By correlating standard water quality measurements with degradation rates, we can predict turnover times for energetics released into hydrodynamically similar unexploded ordnance (UXO)-impacted ecosystems where access to site samples may be limited. Three data gaps are being addressed as part on an integrated sampling scheme of DoD-relevant field sites: 1) determining energetic metabolism rates for ecosystem types where we currently lack data; 2) correlating biogeochemical water quality data with biodegradation rates; and, 3) determining ecosystem capacity for metabolism of energetic compounds. By seasonally repeating these survey measurements and mixing experiments across DoD-relevant ecosystems and further identifying the specific areas and conditions associated with energetic degradation, we will increase the likelihood of public and regulatory acceptance of natural attenuation as a viable risk reduction alternative. Documenting and validating such natural pollution abatement mechanisms supports continued use of active DoD ranges.

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BACKGROUND

Although SERDP, Army and Air Force have sponsored much work on energetic transformation in terrestrial and groundwater systems, relatively little information is available on attenuation or transport of energetics in coastal aquatic systems. In lieu of evidence that these materials are rapidly degraded to nontoxic substances or metabolized by microbial assemblages, DoD is being pressured to recover all UXO from coastal waters and decontaminate surrounding media due to presumed ecological risk (*e.g.,* CH2M HILL 2006). Historically, TNT and other energetics were believed to be recalcitrant to biodegradation to harmless products (*e.g.,* bacterial biomass, CO_2; Hawari et al. 2000, Spain et al. 2000, Claus 2014). However, these conclusions were largely based on bacterial laboratory culture and field work on terrestrial and groundwater sites (*e.g.,* Myers et al. 1998, Travis et al. 2008, Khan et al. 2013) that are unlikely to apply to natural assemblages in coastal estuarine and marine systems (Rappe and Giovannoni 2001). Most energetic compounds that are currently the subject of DoD site investigations are nitrogen-containing organics and organic nitrogen itself is typically scavenged by natural bacteria in coastal ecosystems (Pomeroy 1970, Paerl and Piehler 2008). Anecdotally, energetic organics are not typically found during surveys of UXO-impacted coastal water and sediment which suggests that they may be more transient than the laboratory findings indicate (*e.g.,* CH2M HILL 2000, NOAA 2006, Simmons 2007). A limited amount of further investigation will likely reconcile these disparate findings resulting in cost savings by eliminating unnecessary cleanup and restoration of coastal sediment that is perceived to be (but not actually) impacted by energetics for a length of time that would pose an unacceptable ecological or human health risk.

Recent work by our group and others has focused on determining energetic degradation rates and biogeochemical control of these rates amongst water and sediment of aquatic ecosystems (Conder 2002, Douglas et al. 2009, Zheng et al. 2009, Chappelle et al. 2011, Fahrenfeld et al. 2013) and in biofilms (Zhang et al. 2015). As part of our previous project, ER-1431 (Montgomery et al. 2008), we concluded that TNT biodegrades and photodegrades on ecologically relevant time scales (*e.g.* hours to months) but that other abiotic chemical processes (*e.g.,* hydrolysis) may be too slow. Biodegradation rates of TNT in nitrogen-limited coastal waters are surprisingly rapid compared to rates in terrestrial systems (which are usually phosphorus-limited) and may be the dominant removal pathway for estuarine and marine ecosystems (Montgomery et al. 2011b). TNT mineralization has been reported in many studies to range from 1 to 1000 $\mu g\ kg^{-1}\ d^{-1}$ once data are converted into common units (Montgomery et al. 2011a and references therein). These rates are often similar to those of other natural and anthropogenic aromatic organics with half lives of days to months (*e.g.,* PAHs; Boyd et al. 2008).

TNT ring carbon is incorporated into bacterial macromolecules at rates in oxygenated water that are 10-100 times higher than mineralization rates (complete conversion to CO_2; Montgomery et al. 2013) though incorporation rates have yet to be measured in sediment assemblages because of technical reasons (*i.e.,* high background due to abiotic binding to humic). Adding in TNT incorporation rates as part of total metabolism reduces half lives to hours to weeks (*i.e.,* similar to those rapid rates for amino acids; Kirchman 1994). TNT incorporates into bacterial macromolecules including DNA which enables identification of bacterial genotypes involved in complete TNT biodegradation in natural samples and is relatively unequivocal evidence that

TNT ring carbon is fully metabolized amongst natural sedimentary assemblages (Gallagher et al. 2010). Because TNT is metabolized and the ring carbon incorporated at high efficiency (*i.e.,* low proportion of carbon diverted to CO_2), it is very unlikely that toxic intermediates are produced as part of this metabolic process. This contrasts with what has been shown in soil and groundwater when high concentrations of TNT are used with relatively low bacterial production (*e.g.,* dissimilatory metabolism with TNT used as an alternate electron acceptor verses assimilatory TNT metabolism for biomass production in aerobic coastal waters; Clark and Boopathy 2007, Kubota et al. 2008). Though recently Boopathy (2014) has reported incorporation efficiency of around 25% of TNT ring carbon under sulfate reducing conditions using a natural bacterial assemblage from soil. Because of high TNT incorporation efficiency measured in most coastal waters, it is likely that TNT mineralization rates are actually re-mineralization of radiolabelled bacteria by protozoan grazers (Caron et al. 1988) rather than use of TNT ring carbon for energy rather than biomass (Montgomery et al. 2013).

Organic carbon metabolism rates (including TNT and all other organic energetics) are generally bounded by those of total heterotrophic bacterial metabolism (secondary production; Kirchman et al. 1985). Static, unbioturbated sediment and slow moving, stratified, anaerobic waters will limit biodegradation as bacterial production will likewise be limited. Transitional coastal areas, such as bioturbated, surface sediment (Montgomery et al. 2008), fronts between water masses (Borsheim 1990, Floodgate et al. 1981, Josefson and Conley 1997), tidal salt marshes (Weston et al. 2011) and intertidal zones (Rocha 2008) will often have the highest rates of carbon metabolism. Within the three order of magnitude range of total bacterial production, biogeochemical factors that control TNT biodegradation (and other nitrogenous energetics like RDX and HMX) will likely be those that influence rate of nitrogen metabolism (*e.g.,* nitrogen demand by the assemblage; availability of more labile nitrogen sources) including parameters like salinity, temperature, and dissolved oxygen. In ER-1431, we found that there appeared to be some influence of salinity on energetic degradation. Upon more in-depth examination in ER-2124, we found that it was actually areas of rapid salinity change that were associated with enhanced TNT degradation (Montgomery et al. 2012). Salinity change was indicative of the location of these important frontal boundary regions rather than being a controlling biogeochemical property itself. These are important distinctions when trying to model energetic fate in a natural ecosystem.

Also within the upper bound of total bacterial metabolism are likely factors that preferentially influence aromatic organic carbon degradation. Based on our earlier work, TNT metabolism (also aromatics like DNT, DAT) appears to be influenced by parameters that control degradation of the aromatic component of dissolved organic carbon (DOC; *e.g.,* lignin, PAHs) in the water column and particulate organic carbon (POC) in sediment (Montgomery and Osburn 2004). These biogeochemical features may include presence of more labile carbon (*e.g.,* simple sugars) that would outcompete energetics as a bacterial carbon source.

This research extends our previous SERDP work by examining biogeochemical control of degradation rates in studies of DoD relevant ecosystems where we have a paucity of data. Three data gaps are being addressed as part on an integrated sampling scheme of DoD relevant field sites: determining energetic metabolism rates for *ecosystem types* where we currently lack data;

correlating biogeochemical water and *organic matter quality* data with areas of elevated degradation rates; and, determining *ecosystem capacity* for metabolism of energetic compounds:

Ecosystem Types: Though there are site specific variables that affect each controlling factor at a given site, there are general features that can be used to bound these variables for a given ecosystem type (biome). Navy underwater coastal UXO sites can be categorized for Remedial Program Managers (RPMs) according to expected range of each variable based on published data (Table 1), though in some cases, for historically restricted-access areas, or OCONUS sites of limited published study, empirical data may need to be collected. Published data for similar or nearby ecosystems might be transferable to sites of interest that lack data (Figure 1) or may be used to estimate expected degradation rates involving a known or hypothetical amount of munitions breached at a coastal site (Figure 2). These rates may also be used by RPMs to estimate the likely turnover time for an energetic that has migrated from surface soil of a coastal range and deposited into the surface sediment of an adjacent coastal ecosystem (Table 2).

Table 1. Range of values of biogeochemical factors that influence energetic degradation may be classified according to ecosystem. This may help an RPM classify their site and identify candidates for Monitored Natural Attenuation (MNA) or No Further Action (NFA).

Coastal Ecosystem	Characteristics			Biogeochemical Factors						States/ Territories	Coastal Sites
	Temperature	Rainfall	Sediment Type	Salinity	Sediment Load	DO	DOC	Nitrogen Demand	Bacterial Metabolism		
Arctic	low	high	silt/gravel	---	---	---	---	---	---	AK	Adak, AK-01, AK-2, AK-04, AK-12
Northern Coniferous	low	high	silt/clay	---	---	---	---	---	---	WA, OR, No CA	Ostrich Bay, Jackson Park, Puget Sound, El Toro, Mare Is., Treasure Is., Moffett Field, Crow's Landing, Concord, WA-X01, CA-X01
Northern Temperate	variable	moderate	silt/clay	---	---	---	---	---	---	ME, MA, NH, RI, NY, CT	Portsmouth, Nomans Is. , South Weymouth, RI-01, Fort Constitution Range Complex, Fort Stark Range Complex, Duck Is.
Southern Temperate	variable	moderate	silt/clay	---	---	---	---	---	---	NC, MD, VA, DE	White Oak, Fort Miles, Blossom Point, Camp LeJeune, Cherry Point, Dare County, Dalgren, Wood/Cat Island, Long Shoal, NAAS Edenton, Bull Bay, Drummond Point, Corolla, Duck, Southern Shores, Jockey's Ridge
Subtropical (dry)	moderate	low	sand/silt	---	---	---	---	---	---	So CA	San Diego Bay, Seal Beach, CA-12, CA-03
Subtropical (wet)	moderate/high	high	silt/clay	---	---	---	---	---	---	LA, AL, MS	Offshore LA-X01, LA-X02, LA-02, AL-X01, MS-X01, AL-01
Tropical (dry)	high	low	carbonaceous/ sand	---	---	---	---	---	---	HI (leeward)	Pearl Harbor, Ordnance Reef, Leeward Offshore Disposal Areas HI-01, HI-02, HI-05
Tropical (wet)	high	high	carbonaceous/ sand	---	---	---	---	---	---	PR, FL, HI (windward)	Vieques, Fleming Key, Trumbo Point, Sigsbee Park, North Boca, Dry Tortugas

Organic Matter Quality: It is likely that establishing a correlation between metabolism of TNT and aromatic organic carbon may be the best predictor of TNT removal by natural bacteria in surface water and sediment. Unfortunately, it is also the most difficult to establish directly because there are few radiotracers for natural aromatic organics (*e.g.,* ^{14}C-lignin monomers > $60K per experiment). The best way to examine this may be statistical analyses of changes in DOC quality or character (*i.e.,* aromaticity, humification) as measured by fluorescence (Osburn et al. 2012). If this correlation can be established, it may be possible to model TNT metabolism rates of large sites using *in situ* instrumentation deployed by personnel or by remote observatories (*e.g.,* AUV, UUV) while minimizing the need to sample submerged sites amongst hazardous UXO and obviating expensive chemical analyses.

Ecosystem Capacity In addition to using energetic biodegradation rates to calculate natural attenuation rates of energetics already present in an ecosystem (based on the biome type above), one can also use this data to calculate ecosystem capacity for degrading compounds yet to be released into coastal waters. This calculation would be important for determining how much energetics or PAHs that an active range could release into an ecosystem before there was a high likelihood of ecological damage. A range manager could then alter the amount or schedule of range activity to mitigate potential negative impacts based on known influencing factors (*e.g.,* seasonal residence time, temperature, rainfall).

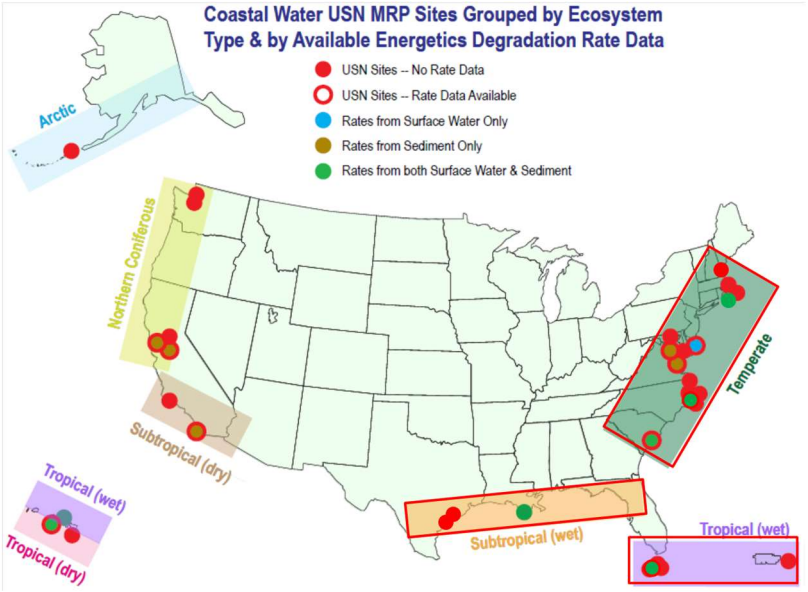


Figure 1. Coastal USN Munitions Response Program sites grouped by ecosystem type with designations for those that have no corresponding data for rates of energetics degradation ( ). Sites with data have a red border ( ) that is filled based on type of media for which the data available: surface water ( ); sediment ( ); or both ( ). Additional sites where data are available but with no nearby MRP site are designated without the red outline.

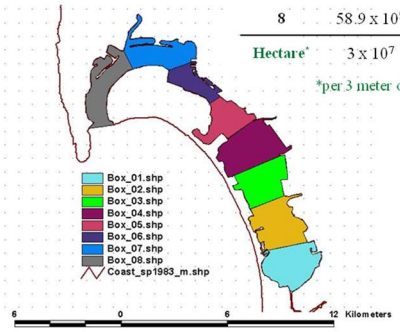
Table 2. As an example of how mineralization rates can be used by an RPM for site evaluation, if 1 ppm of TNT is found in surface soil of a coastal range and this soil is deposited onto the surface sediment (10 x 100 x 1 cm deep = ca. 1 kg) of an adjacent subtropical (wet) coastal ecosystem, one would predict that turnover time for this flux of TNT would be 22 h to 10 days based on measured range of TNT mineralization for that ecosystem. Rates in blue from current project.

MEC (Organic N)	Aromatic	Reported Degradability (relative to TNT)	Coastal Ecosystem Rates (µg kg^{-1} d^{-1})						
			Arctic	Northern Coniferous	Temperate	Subtropical (dry)	Subtropical (wet)	Tropical (dry)	Tropical (wet)
TNT	Yes	--	---	0.05-7.4	0.04 – 270	0.08-0.204	0.1–0.9	2-52	0.38–95
DNT	Yes	same	---	16 – 68	0.8 – 220	---	---	---	---
DAT	Yes	lower	---	---	1.4 – 49	---	---	---	---
RDX	No	same	---	---	0.64 – 884	---	46	---	0.25 – 690
HMX	No	lower	---	---	1.1 – 75	---	10	---	0.14 – 50
Nitroglycerine	No	higher	---	---	---	---	---	---	---

Capacity of a water body to naturally biodegrade TNT suddenly released from a breached ordnance varies with ecosystem but can be substantial (e.g. size of SD Bay = 100-3800 shells yr^{-1})

No.	Water Volume (L)	TNT Mineralization Rate (AVG (SD), kg yr^{-1})			Capacity to Degrade Amount of TNT in Breached 24 inch Shells (AVG(SD), shells yr^{-1})		
		Temperate	Subtropical	Tropical	Temperate	Subtropical	Tropical
1	12.5×10^9	1333 (542)	42 (21)	625 (229)	171 (71)	5 (3)	75 (29)
2	22.1×10^9	2357 (958)	74 (37)	1105 (405)	302 (125)	9 (5)	133 (52)
3	26.9×10^9	2869 (1166)	90 (45)	1345 (493)	368 (152)	11 (6)	161 (63)
4	44.6×10^9	4757 (1933)	149 (74)	2230 (818)	610 (253)	18 (10)	268 (104)
5	28.4×10^9	3029 (1231)	95 (47)	1420 (521)	388 (161)	11 (7)	170 (66)
6	28.7×10^9	3061 (1244)	96 (48)	1435 (526)	392 (163)	11 (7)	172 (67)
7	55.4×10^9	5909 (2401)	185 (92)	2770 (1016)	757 (314)	22 (13)	332 (129)
8	58.9×10^9	6283 (2552)	196 (98)	2945 (1080)	805 (334)	24 (14)	353 (137)
Hectare*	3×10^7	3.2 (1.3)	0.10 (0.05)	1.5 (0.55)	0.41 (0.17)	0.012 (0.007)	0.18 (0.07)

*per 3 meter of water depth



Length (inches)	Diameter (inches)	Hole radius (mm)	MC Mass (kg)
24	8	20 mm	8

San Diego Bay model from ER-1453 (Wang, Lao, George & Wild 2008).

Figure 2. TNT degradation rates can be presented in terms of amount of time it would take to attenuate a breached munition in a coastal environment. Here is an example of using our TNT mineralization rates in a transport model developed in SERDP ER-1453 for San Diego Bay.

MATERIAL AND METHODS

Site and Sampling Description Over the course of this three year project (CY13-15), ten field samplings were undertaken along with three samplings in collaboration with a mesocosm experiment with ER-2122 (Table 3). In Year 1 (CY13), a wet tropical reef and lagoon system in the Florida Keys was sampled three times during 13-16 May, 5-7 August and 13-16 November using a 22' Sea Chaser boat. The survey of coastal NAVFAC sites on Key West (Fleming Key, Trumbo Point, and Sigsbee Park) and Boca Chica Key (North Boca) was coupled with a study of lagoons on No Name Key (Figure 3) that are analogous to those found on Vieques Island, Puerto Rico (Figure 4). The survey, which included four NAVFAC sites, was coordinated with RPMs Dana Hayworth and Brian Syme at NAVFAC SE Atlantic IPT. The November 2013 sampling included a large wind event that limited the survey due to small craft advisories. In year 3 (CY15), a fourth Key West sampling was undertaken 20-26 October though the offshore sampling was again limited (one mile SW of Southermost Point) because of small craft advisories.

Also in CY13 and CY14, a saltwater marsh mesocosm was sampled in coordination with ER-2122 (Dr. Craig Tobias: *Tracking the Uptake, Translocation, Cycling, and Metabolism of Munitions Compounds in Coastal Marine Ecosystems using Stable Isotopic Tracer*) at University of Connecticut during extended experiments in August, September-October, 2013 and June-July 2014. Separate mesocosms were maintained with added TNT or RDX over a several week period. Subsamples of water, foam and sediment were sent to NRL for concurrent measurement of TNT and RDX mineralization. These ^{14}C radiotracer rate measurements were used as a

separate line of evidence of the TNT and RDX attenuation rates determined from chemical loss and mineralization using stable isotopic tracers (*i.e.,* ^{13}C, ^{15}N).

Table 3. Summary of sampling events, number of stations, fronts and experiments from CY13-15.

Location	Dates	Stations	Mixing Experiments	Front Survey	Notes
Key West	13-15 MAY 2013	6	mangrove/ocean	none	
	5-7 AUG 2013	10	mangrove/ocean	sargassum	
	13-16 NOV 2013	2	mangrove/ocean	none	large wind event
	20-26 OCT 2015	10	mangrove/ocean	none	wind event
ER-2122 Mesocosm	AUG 2013	8	N/A	N/A	
	SEP-OCT 2013	8	N/A	N/A	
	JUN-JUL 2013	7	N/A	N/A	
North Carolina	14-17 APR 2014	14	none	creek/sound	DICERP sampling
	4-7 AUG 2014	13	Cypress bog/bay	inlet/ocean	large rain event
	13-17 NOV 2014	13	Cypress bog/bay	river/bay	
	11-18 AUG 2015	23	Cypress bog/bay	turbidity	
Delaware	24 SEP 2014	2	marsh creek/bay	none	
Corpus Christi	16-22 SEP 2015	5	river/mangrove/bay	none	wind/red tide

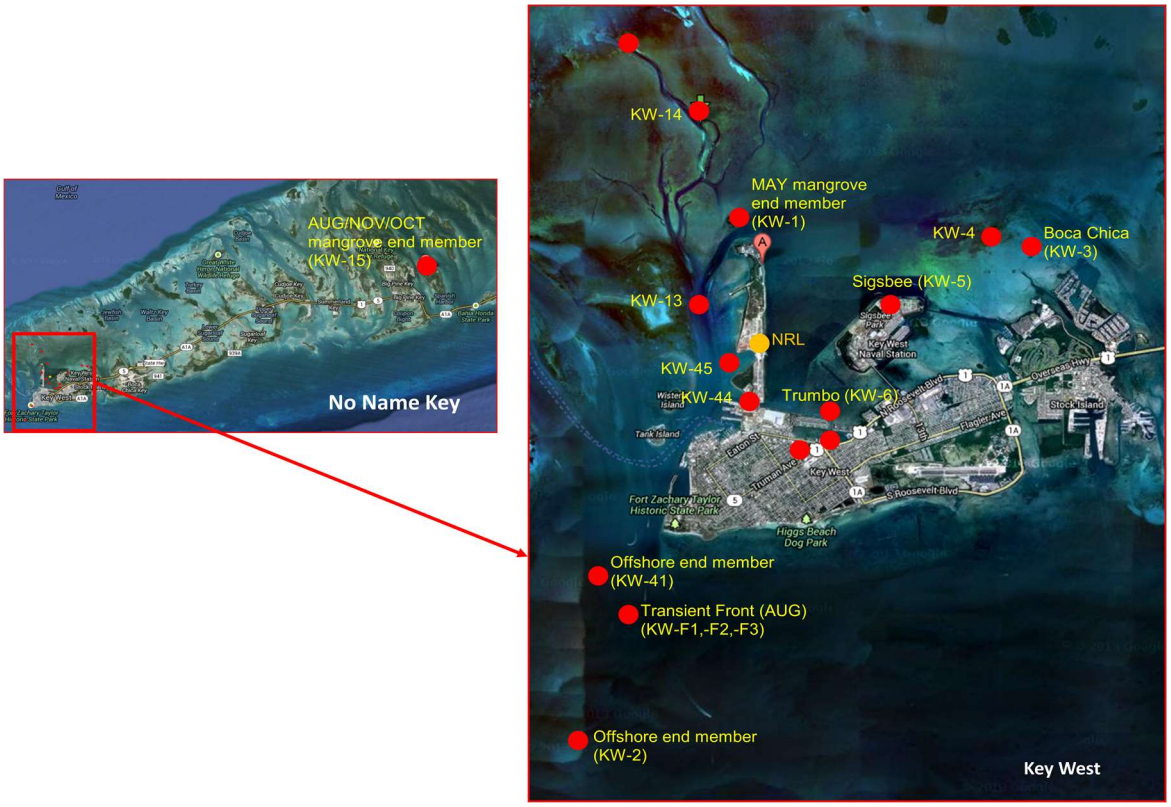


Figure 3. CY13 and CY 15 sampling locations around Key West and No Name Key, Florida, USA (Google Maps).

During year 2 (CY14), Lower Outer Banks, North Carolina, USA was sampled three times over 14-17 April, 4-7 August, and 14-17 November using a 16' Landau Center Console fishing boat. The survey of this southern, temperate lagoon estuary included areas adjacent to three DoD sites in this ecosystem but did not include samples from within actual site boundaries (Cat Island aka Wood Island, Cherry Point, Camp Lejeune; Figure 5). The April sampling included a survey taken alongside UNC scientists working on the SERDP DICERP assessment of the New River adjacent to Camp Lejeune (Figure 6). August and November samplings included a salinity transect of the Newport River after relatively high (August) and moderate rainfall (November) events (ca. 12 and 5 inches, respectively). All three of these sampling events included opportunistic sampling of transient frontal boundaries between a creek and Bogue Sound (April), Beaufort Inlet and offshore (August), and Cypress bog and Newport River (November). All three samplings included mixing experiments between the Cypress (pocosin) bog water and adjacent Newport River water or offshore samples. During the November sampling, effect of statin (Provect-CH4, 50 ppm final concn.) was measured across a Newport River salinity transect. Effect of the bioremediation supplement, Provect-IR (75 mg mL^{-1} wet sediment), on carbon substrate mineralization rates was also measured using surface sediment from Bogue Sound.

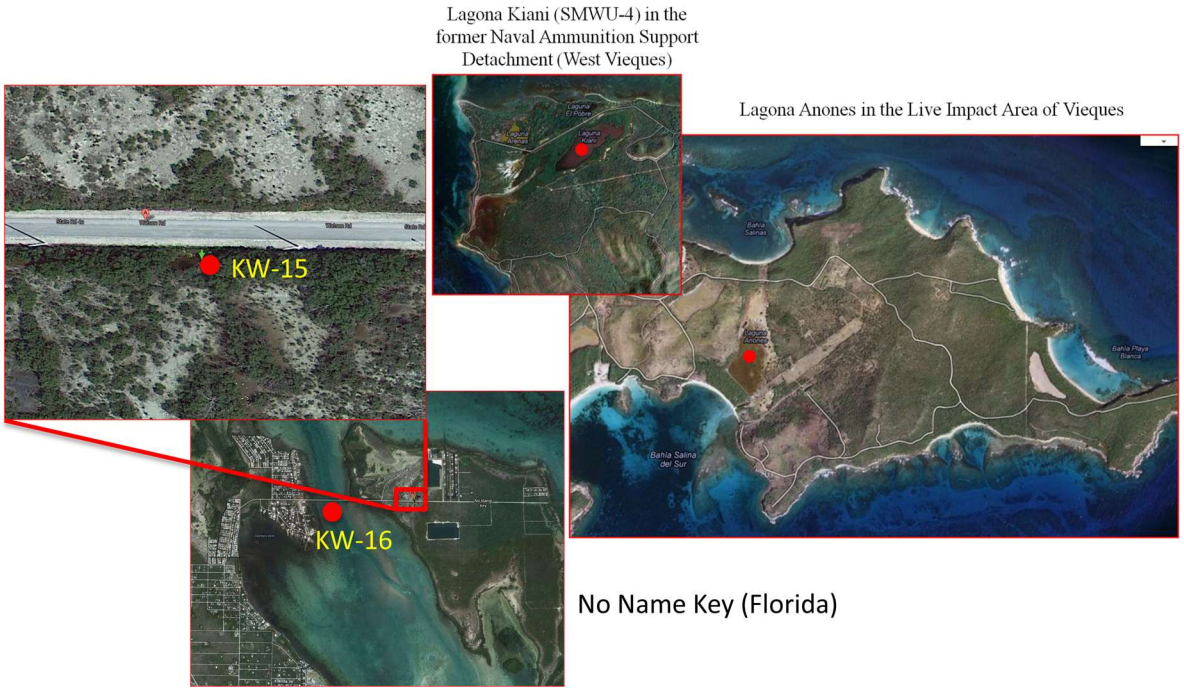


Figure 4. Potentially UXO-impacted lagoons on Vieques Island, Puerto Rico, USA. Study area at No Name Key (KW-15) was chosen to approximate less accessible areas in these other subtropical ecosystems. November mixing experiment included water from the adjacent waterway (KW-16; Google Maps).

Figure 5. CY14 and CY15 sampling locations around Lower Outer Banks, North Carolina, USA (Google Maps).

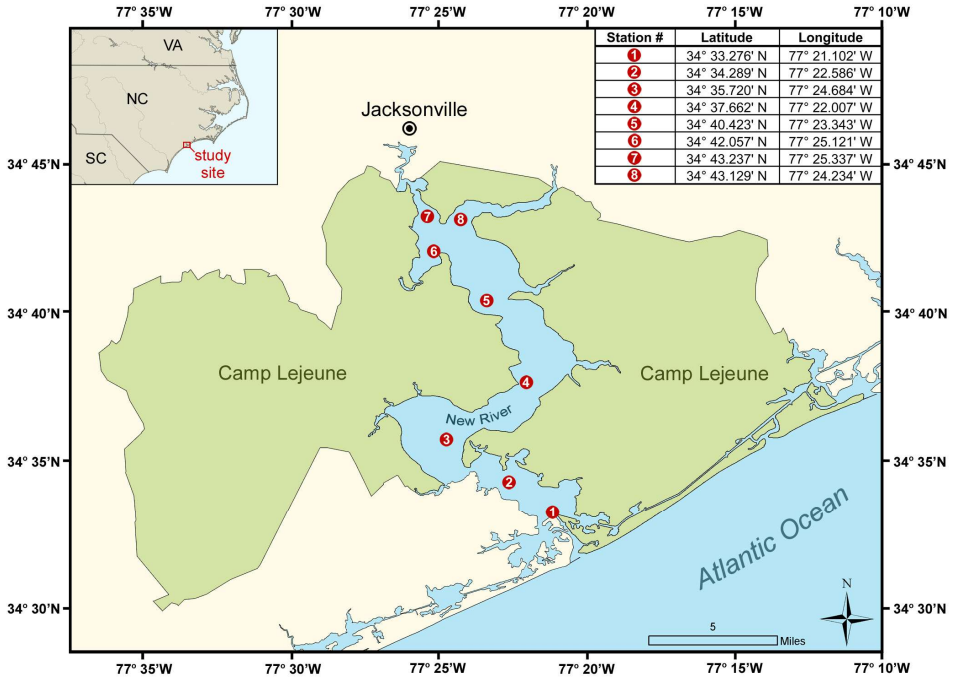


Figure 6. New River Estuary, NC, USA, DICERP sampling locations during April 2014 survey. Note DICERP station numbers are in reverse order from those of this project (Courtesy of Hans Paerl, 2015).

In year 2 (CY14; 24 SEP 2014), a limited sampling and mixing experiment was performed with two stations taken from Canary Creek headwaters (DE-2; 25 PSU) and confluence of Canary Creek with Roosevelt Inlet (DE-1; 27 PSU) in the Great Marsh Preserve, DE, USA (Figure 7). In addition to the mixing experiment, effect of a statin, which is an archaebacterial cell wall inhibitor (Provect-CH4; 50 ppm final concn.), and a commercial biodegradation-stimulating amendment (Provect-IR, 50 ppm final concn.; Provectus Environmental Products, Freeport, IL) were tested for their effect on heterotrophic bacterial production and carbon substrate mineralization. In addition, to differentiate between the importance of the bacterial assemblage

of each end member to total bacterial production, the production assay was performed on (90:10) mixes of whole water from DE-1 and either whole water of DE-2 or filtered (0.22 μm nom. pore dia.) water from DE-2.

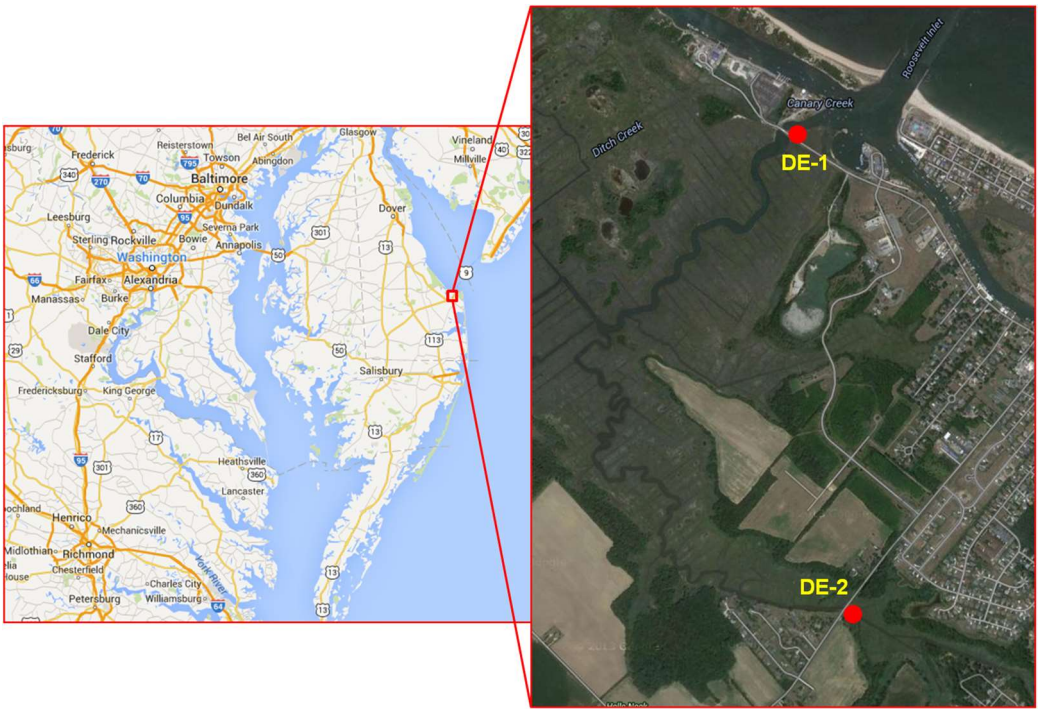


Figure 7. CY14 (24 September) sampling locations around Great Marsh Preserve, Lewes, Delaware, USA (Google Maps).

In year 3 (CY15), there was one sampling each at the Lower Outer Banks, NC (11-18 August), Corpus Christi Bay, TX (16-22 September), and Key West, FL (13-20 October). During the Lower Outer Banks field work, the Newport River and Bogue Sound surveys and mixing experiments were repeated. Additional stations were taken in the Neuse and White Oak Rivers. Perhaps the most distinctively defined turbidity front that we encountered during the entire project was sampled off Bear Island at the confluence of the White Oak River and Bogue Sound.

The lagoon estuary adjacent to Corpus Christi, TX, represents an ecosystem with relatively high petroleum input but where there were no published rates of energetics biodegradation (Figure 8). Station collection was limited due to high winds but several were gathered from the Oso River, Corpus Christi Bay and offshore near Port Aransas Pass. High rains and a red tide preceded the sampling event. Separate mixing experiments were performed between offshore water (CC-2) and either the eutrophic Oso River (CC-1) or a mangrove flat near Port Aransas (CC-4).

The final sampling took place around Key West, FL, but was also limited (small craft advisory) to the offshore station (one mile SW of Southernmost Point, Key West; KW-41), the NAVFAC survey (e.g., Trumbo Point, Sigsbee Park) and the No Name Key mangrove lagoon (for the standard mixing experiment with the offshore sample).

Typically, water column (using CTD, Niskin bottles) and sediment samples (benthic grab, Wildco) were collected along salinity transects of these coastal systems with sampling concentrated across fronts between water masses and salt wedges. Standard water quality

measurements of dissolved oxygen (DO), salinity (practical salinity units, PSU), temperature (°C), pH were made with a hand-held YSI Multiprobe™.

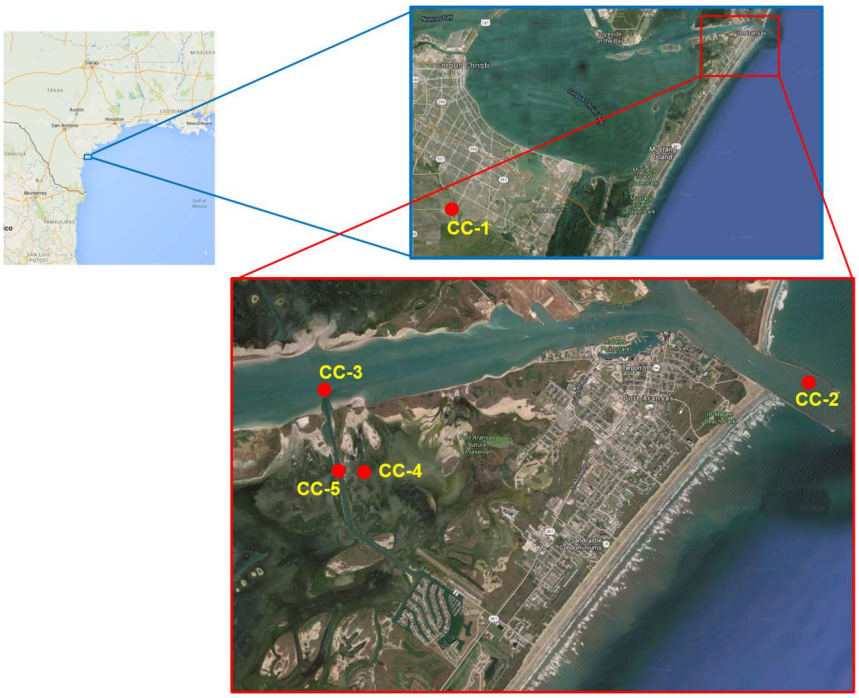


Figure 8. CY15 sampling locations around Corpus Christi, Oso and Baffin Bays, Texas, USA (Google Maps).

TNT, RDX, HMX, and Phenanthrene Mineralization Rates of bacterial metabolism of aromatic contaminants were measured by mineralization of ^{14}C-radiolabelled substrates to $^{14}CO_2$ which are particularly useful for evaluating contaminant biodegradation in field studies (Fenner et al. 2013). These assays were typically initiated within 2 h of sediment sample collection using a modification of Boyd et al. (1996) and Pohlman et al. (2002). Carbon substrates 2,4,6-TNT [ring-^{14}C(U)] (4 mCi mmol^{-1}, American Radiochemical Corporation, 99% purity), 9-^{14}C-phenanthrene (PHE; 55.7 mCi mmol^{-1}), UL-^{14}C-RDX (1.13 mCi mmol^{-1}, Defence R&D Canada), and UL-^{14}C-HMX (1.97 mCi mmol^{-1}) were added in separate incubations to 100 x 16 mm polycarbonate test tubes (ca. 0.2 µg g^{-1} or 0.04 µg mL^{-1}, depending on specific activity). For sediment, 0.5 mL of bottom water from the same station was filtered (0.22 µm nom. pore dia., Millipore) and added to make slurries with sediment that was cored from surface of the benthic grab sample using a five-mL syringe with the end cut-off. Triplicate live and one kill (2 mL of 2 N H_2SO_4) of all samples were incubated for ca. 48 h at *in situ* temperature in the dark and evolved $^{14}CO_2$ captured on NaOH-soaked filter papers. H_2SO_4 (2 mL, 2 N) was likewise added to end live incubations and to partition any remaining CO_2 into headspace of the tube and to the filter paper trap. Filter paper traps containing metabolized $^{14}CO_2$ were removed, radioassayed and subsequently used to calculate substrate mineralization. Triplicate one-mL syringed samples of wet sediment were dried (overnight; 50°C) and used to convert mineralization values sample into kg sediment dry weight. Detection limit of the assay was typically 0.01 µg C L^{-1} d^{-1} though average values that were below one standard deviation were considered below detect (0, BD).

Bacterial Production Growth rate of the heterotrophic bacterial assemblage (in terms of carbon) was measured by the leucine incorporation method of Smith and Azam (1992) for water

(1.0 mL) or wet sediment (50 µL) as adapted by Montgomery et al. (2010). Environmental samples from each station were added to 2.0 mL microcentrifuge tubes (three experimental and one killed control) sealed with a cap with an O-ring that was pre-charged with [^{3}H-4,5]-L-leucine (120 mCi mmol^{-1}, final concn. 20 nM). Sediment was extracted from the sample using a one-mL polypropylene syringe with the end cut off and then extruded to the assay tube. For sediment, 1.0 mL of 0.22 µm (nom. pore dia.) filtered bottom water was then added to each tube and vortexed to form a sediment slurry. All samples were incubated at *in situ* temperature for 30-60 min. Incubations were ended by adding 57 µL of 100 % trichloroacetic acid (5 % final concentration; TCA, Fisher Scientific) and frozen for storage prior to processing by the method of Smith and Azam (1992). Killed controls have the TCA added prior to sample addition and these values were subtracted from those of the experimental samples. A constant isotope dilution factor of two was used for all samples and was estimated from sediment dissolved free amino acids measurements (Burdige and Martens 1990) and saturation experiments (Tuominen 1995). Triplicate one-mL syringed samples of wet sediment were dried (overnight; 50°C) and used to convert sediment production values into dry weight. Leucine incorporation rate was converted to bacterial carbon using the formula of Simon and Azam (1989). Assay detection limit was 1.0 µg C kg^{-1} d^{-1} though average values that were below one standard deviation were considered below detect (0, BD).

Bacterial Organotolerance Inhibition of bacterial production by naphthalene (organotolerance) was measured as a proxy for semivolatile organic or osmotic stress at salinity fronts. Naphthalene organotolerance of the bacterial assemblage was measured by adding 0, 5, 10, 15 or 25 µg of naphthalene in methanol (5 µL) to 0.50 µL of wet sediment or 1.0 mL of water and subsequently processed for bacterial production (Montgomery et al. 2010). All treatments and controls received the same addition of methanol (5 µL) though previous experiments showed that production was not affected in parallel incubations with this amount of methanol alone (Montgomery et al. 2010). The 5 µL of methanol with dissolved naphthalene was added to each microcentrifuge tube prior to sample addition. Average and standard deviation of three live incubations (with value for killed control subtracted) was regressed to amount of naphthalene added to the leucine incorporation assay. Final concentration of naphthalene in the 25 µg addition was ca. 250 µg g^{-1} sediment (dry weight) and 25 µg mL^{-1} of water. The regression formula and r^2 value were calculated using Microsoft Excel®.

DOC Analyses DOC was quantified by wet chemical oxidation on 2 mL sample volumes, using concentrated and cleaned sodium persulfate (Osburn and St-Jean 2007). Limit of detection via this method was 12 µmol L^{-1} C and reproducibility was <5%. Potassium hydrogen phthalate was used as a calibration standard for DOC concentrations over a range of 83 to 1,666 µM. DOC stable isotope values (δ^{13}C-DOC) were measured on samples and calibrated to the international PDB scale with sucrose (δ^{13}C=-10.45‰) and caffeine (δ^{13}C=-27.77‰) obtained from NIST and/or IAEA. δ^{13}C-DOC values indicate organic matter source with values of -27 to -25‰ indicating terrestrial (and presumably more aromatic) organic matter, while marine phytoplankton range from -22 to -19‰. Salt marsh plants such as *Spartina spp.* generally are isotopically enriched with values between -11 and -14‰.

Lignin Analyses Presence and degradation state of terrestrially-derived OM (*i.e.,* lignin) was determined by measuring lignin concentration and determining its relative degree of oxidative

degradation. Lignin was measured as its component acid, aldehyde, and ketone phenols after microwave assisted CuO-oxidation (Louchouarn et al. 2000, Goñi and Montgomery 2000). Phenols were extracted into ethyl acetate, redissolved into pyridine, derivatized, and then analyzed by GC/MS on a Varian 431-220MS using a DB-5 ms column. Lignin phenols were quantified against a standard curve of each of eight phenols released during the oxidation procedure (vanillin, vanillic acid, acetovanillone, syringealdehyde, syringic acid, acetosyringone, p-courmaric acid, ferulic acid). As there is currently no accepted radiotracer method for measuring bacterial lignin metabolism, ratios of vanillic acid to vanillin ($Ac:Al_v$) content were used to indicate oxidative degradation (Hedges and Mann 1979).

Absorption and Fluorescence Spectroscopy Relative aromatic character of refractory dissolved organic carbon (DOC) was measured by its absorptive and fluorescent properties. Spectral absorption (200-800 nm) was measured on a Varian Cary 300UV spectrophotometer and excitation-emission matrix (EEM) fluorescence on a Varian Eclipse spectrofluorometer on 0.2 μm (nom. pore dia.) filtrates from water samples. EEM fluorescence was measured on filtrates for dissolved organic matter (DOM) and on 0.1 N NaOH extracts of 0.7 μm GF/F filters for particulate organic matter (POM) fluorescence (Osburn et al. 2012); the appropriate Varian Eclipse instrument corrections applied and fluorescence data were reported in Raman-normalized quinine sulfate equivalents (QSE) in ppb, corrected for sample absorption (*i.e.,* inner filter effects). All EEM data were modeled using the Parallel Factor Analysis (PARAFAC) procedure to decompose the fluorescent matrices into fluorescent components (Stedmon and Markager 2005, Stedmon and Bro 2008). Optical data processing was performed using Matlab software.

Demarcation of Salinity Fronts, Salt Wedges and Confluence Zones We used change in salinity over relatively narrow spatial scales to identify an area as a front, wedge or zone of confluence. Collection bottles that contain water samples whose salinity is intermediate between surface water at the beginning of the rapid change and at the end near that of bottom water (in the case of a salt wedge) were considered representative of the frontal boundary sample. Collection of samples from convergence zones that interface at the air-sea boundary (*e.g.,* horizontal gradient) is more straightforward as these areas are typically catchments for flotsam and detritus that can be clearly seen from aboard the boat deck. Radon isotope methods may also be used for determining the age of adjacent water masses relative to the front to estimate entrainment time of a water parcel at the frontal boundary (*e.g.,* Moore 2000, Charette et al. 2001, Hougham and Moran 2007, Peterson et al. 2008) though a proposal to use this strategy in year 3 was not adopted by the SERDP committee.

Mixing Experiments Mixing experiments were performed to examine the effect on bacterial production, aromatic degradation and DOC properties by mixing different water mass end members in a 1 L polycarbonate bottle in the dark. The purpose of these experiments was to simulate the biogeochemical conditions and changes that occur at frontal boundaries when water masses first begin to mix in an estuary. An advantage of this method verses simple estuarine surveys of frontal boundaries is that the time from the start of mixing is known. All mixing experiments involved 1 L of each end member from a mangrove effluent sample and an open water sample. The first two samplings involved a single 50:50 mix of end members (May, KW-1, -2; August, KW-15, -2), whereas the November sample involved 5 mixtures as a percentage of mangrove end member (KW-15) and a nearby open water end member (KW-16; 10%, 20%,

30%, 40% and 50%). Treatments were subsampled for bacterial production, contaminant mineralization and DOC at the start of the incubation (T_0) and for bacterial production and DOC at incubation end (T_f: May, 65 h; August, 65 h; November, 94 h). Mineralization rates were only determined for the T_0 mix and end members because the assay incubation time was different than that for bacterial production (>48 h vs 1 h).

The October 2015 Key West mixing experiment involved the No Name Key mangrove bog end member (KW-42, previously KW-15) and the offshore end member (KW-41) that was ca. one mile SW of Southernmost Point. Mixes includes 0.1, 1, and 5% mangrove lagoon water diluted into the offshore end member. Additional treatments were examined where radiolabel-charged tubes were pre-incubated with the mangrove addition for one hour prior to the mixing experiment. This setup was designed to determine if increasing the initial encounter time between the mangrove assemblage (unfiltered), and/or possibly the humic chemical component of the mangrove effluent (filtered), would affect substrate mineralization rate. Humic micelles may coat the aromatic substrate (sterically hindering the binding to the cell surface receptor protein) and reduce substrate mineralization depending on how long it would take to disassociate the humic from the substrate upon dilution with seawater (below the critical micelle concentration).

Lower Outer Banks, NC, mixing experiments followed a similar strategy to those of Key West except they involved a Cypress bog freshwater end member rather than that of a mangrove lagoon. No mixing experiment was performed during the April 2014 sampling but the August sampling mixed the Cypress bog (pocosin bog, Newport, NC; NC-47) with an offshore end member (0, 1, 2, 5, 10, 25, 100%; NC-40). The offshore end member was used during this high flow storm event because lower salinity water was pushed farther outside Beaufort Inlet. During the moderate flow sampling event in November 2014, that same Cypress Bog freshwater end member (NC-66) was mixed with Beaufort Inlet water taken adjacent to Radio Island (0, 1, 2, 5, 100%; NC-67). In addition, a mixing experiment was performed during the November sampling to determine relative importance of the bacterial assemblage associated with the Cypress bog verses that of the marine end member. Mineralization in mixes could be stimulated by high nutrient addition to the offshore assemblage which would be seen as enhanced rates in the incubation with 99% unfiltered seawater mixed with 1% Cypress bog filtrate. Mineralization by the Cypress bog assemblage may also be enhanced by dilution of some inhibitory substance (*e.g.,* humics) present at high concentration in the bog end member which would be seen as enhanced rates in the incubation with 99% seawater filtrate mixed with 1% Cypress bog unfiltered water.

A final Lower Outer Banks mixing experiment was performed using Cypress freshwater bog end member (NC-108) and an offshore end member (NC-107 off Beaufort Inlet). Mixes included 0.1, 1, and 5% Cypress bog water diluted with offshore seawater, as well as, a set where the isotope was pre-incubated (50 min) with the bog water as described for the final Key West mixing experiment.

Two mixing experiments were performed during the August 2015 Corpus Christi, TX, sampling and incolved mixing the offshore end member (CC-02; 31.95 PSU) from Aransas Pass with either the freshwater end member in the Oso River (CC-01, 2.76 PSU) or the hypersaline,

mangrove flat end member (CC-04, 41.86 PSU). Both mixing experiments used 0.1, 1, and 5% of high organic end member diluted into the offshore seawater (CC-02).

In September 2014, a simple mixing experiment was performed using the effluent from a *Spartina* marsh grass creek (DE-2, 25 PSU) mixed 1:9 with Delaware Bay seawater (DE-1, 27 PSU) as part of a four day incubation.

Bioremediation Amendment Addition The mixing experiments raised the question as to which component of the natural microbial assemblage was most important for aromatic and energetic biodegradation. In addition to experiments involving removal of each end member assemblage via filtration, statin additions were used to ascertain the role of Archaebacteria in either direct metabolism of energetics or in competition with energetics biodegraders for nutrient resources (thereby reducing mineralization rates). Statins are a class of archaeal cell wall inhibitors that should selectively reduce the role of Archaebacteria in biogeochemical function of the natural heterotrophic microbial assemblage (see review by Liu et al. 2011). The statin amendment, Provectus-CH4 (active ingredient, lovastatin), as well as, a bioremediation amendment containing the statin, Provectus-IR, were provided gratis by Provectus Environmental Products (Freemont, IL). Provectus-IR is currently being used to enhance organic contaminant biodegradation in soil and groundwater (Mueller et al. 2014, Peale et al. 2015). Effect of Provectus-CH4 (10-125 ppm, DE; 50 ppm, NC) and Provectus-IR (50 ppm, DE; 75 mg g^{-1} sediment, NC) was tested on both overall heterotrophic bacterial production and contaminant mineralization during the DE salt marsh sampling (water sample DE-2; September 2014) and the third NC sampling in Bogue Sound (sediment samples NC1-3; November 2014).

Data Interpretation We expected that if there is no effect of water mass interface on TNT metabolism, then the relationship between salinity and TNT metabolism (*i.e.,* mineralization) would be conservative. That is, it would not be different from a linear mixing of end members with respect to salinity or DOC. When there was metabolism different from that which was expected from conservative mixing, this was seen on a graph comparing these parameters with salinity or as a percentage mixture of two end members. Those biogeochemical parameters that could be measured on similar spatial and temporal scales (*e.g.,* DOC fluorescence, bacterial production, etc.) and that demonstrated a similar pattern of change with respect to salinity were considered candidates for further study, as they were putative factors that control TNT metabolism in nature. Ultimately, the data was used to determine the likelihood of an energetic release impacting adjacent coastal waters and capacity of this ecosystem to attenuate these compounds.

RESULTS AND DISCUSSION

This limited scope project primarily focused on demonstrating that frontal boundaries have enhanced rates of TNT mineralization, bacterial metabolism (*i.e.,* heterotrophic production) and degradation of refractory OM (*e.g.,* TNT, PAH, lignin). In addition, study of biogeochemical features at the interface that control these microbial rates and what cellular changes in the microbial assemblage occur in these environments (*e.g.,* organotolerance; Montgomery et al. 2010) was initiated. If TNT metabolism rate can be related to heterotrophic production or OM transformation, then knowledge of general metabolism and carbon cycling rates may be applied

to modeling energetic attenuation and ecosystem capacity to attenuate these compounds from a shoreside release. *Mixing experiments* between water mass end members, samplings of coastal and estuarine *fronts*, and coastal *survey*s were performed to address this topic. It is possible that ambient energetics biodegradation rates alone (without enhancement by frontal boundaries) will give the ecosystem enough capacity to attenuate most firing range input of energetic. These findings on energetic and phenanthrene biodegradation will be discussed with respect to recent literature regarding recalcitrant organic matter biodegradation (Marin-Spiotta et al. 2014). The emerging understanding of biodegradation of what were previously believed to be persistent organics has reinforced our SERDP work here and over the previous 20 years and have dramatic implications for engineered bioremediation field technologies.

Mixing Experiments Variations of a mixing experiment were performed during each of four Key West sampling events (May, August, November 2013, October 2015). The concept was to mimic a natural bacterial assemblage response to a mixing event that would occur between relatively high organic mangrove lagoon water and lower organic open ocean water. In nature, this may occur with tidal flushing or from a rain event that overwashes the lagoon mixing in water from the adjacent coastal ecosystem. Contaminant biodegradation in mangrove-dominated lagoons has been a largely ignored field despite the importance of these systems to the local ecology (see review by Bayen 2012, Tait et al. 2016). The first mixing experiment (May 2013) involved mangrove effluent from amongst the root system that was accessible by boat (KW-1, Figure 9) and was likely to be frequently mixed with adjacent Gulf of Mexico water via wave action. The open ocean end member (KW-2, Figure 9) was collected from ca. four miles south of Key West, FL near Point of Rocks in the Gulf. The second mixing experiment (August 2013) also involved this open ocean end member, KW-2, but the mangrove end member was collected from a much more isolated water body within a mangrove lagoon at No Name Key (KW-15, Figure 9). This sample appeared to have a much high concentration of colored dissolved organic matter (CDOM, Figure 10) and may be more representative of the type of mangrove lagoons impacted by UXO at Veiques, Puerto Rico. These types of lagoons are more likely to be flushed episodically with extreme rain events, such as hurricanes. The third mixing experiment (November 2013) also involved the No Name Key mangrove end member, KW-15, but the open ocean station (KW-2) was inaccessible so water from the adjacent channel (a few blocks away) was collected from the Old Wooden Bridge between No Name Key and Long Key and used as the open ocean end member (KW-16, Figure 9). The latter mixing experiment (KW-15/16) may have better represented the response of the natural assemblage to an overwash of No Name Key lagoon than using the open ocean end member (KW-16 was a block away whereas KW-2 was ca. 20 miles away). The fourth mixing experiment (October 2015) also involved the No Name Key mangrove end member (KW-15) but (because of weather conditions) the offshore end member (KW-41) was collected only one mile south of the Southernmost Point rather than three miles further as was collected during the May sampling.

During the May 2013 survey (no rainfall during the week prior to sampling), mixing the mangrove effluent from Fleming Key with that of the offshore sampled did little to stimulate overall bacterial growth (5% increase), TNT or RDX mineralization, however, phenanthrene mineralization was stimulated by about 58% over that predicted from the end member values (Table 4). HMX mineralization may also have been stimulated but the high standard deviation made this questionable. Note that mangrove effluent from this mixing experiment was likely to

be more frequently mixed with adjacent Gulf of Mexico water than those mangrove lagoon samples used in subsequent mixing experiments.

Figure 9. Mixing experiment stations for lagoon end members near Fleming Key (KW-1), No Name Key (KW-15) and open water end members offshore of Key West (KW-2) and between No Name and Long Keys (KW-16).

Table 4. Rates of bacterial production and mineralization of TNT, phenanthrene (P), RDX and HMX (AVG (SD) µg C L^{-1} d^{-1}) for a mixing experiment between mangrove effluent from No Name Key (KW-1) and offshore of Key West (KW-2; May 2013).

	Rate (AVG (SD) µg C L^{-1} d^{-1})				
	Bacterial	Mineralization			
Station	Production	TNT	P	RDX	HMX
KW-1	67 (6.7)	0	0.85 (0.36)	0	0.14 (0.02)
Mix KW-1/2	42 (1.9)	0	1.46 (0.48)	0	0.65 (0.63)
KW-2	13 (2.0)	0	1.0 (0.06)	0	0

As a result of the May data, during the August 2013 survey (0.27" rainfall during the sampling but none the week prior), it was decided to use mangrove effluent from a more enclosed lagoon that may have more significant water quality differences than between KW-1 and KW-2. No Name Key mangrove lagoon was mixed with the same offshore sampling location end member (KW-2) and showed little positive effect on phenanthrene mineralization (5%) and mixing may have actually depressed mineralization of TNT and RDX (Table 5). It should be noted that the general mixing effect on bacterial production may have been a simple function of oxygen as DO in the mangrove end member was 31%. Subsequent mixing experiments using these end members focused greater proportions of oxygenated offshore water to try and investigate this aspect of the mixing experiment. One measure of a bacterial assemblage's sensitivity to membrane destabilization as a result of osmotic or organic stress is the effect of increasing

concentrations of naphthalene on bacterial production (*i.e.,* organotolerance). Though the mangrove assemblage had much higher bacterial production than that offshore (KW-2), it turns out that the mangrove assemblage was also much more sensitive to naphthalene addition (Figure 11). This suggests that the offshore assemblage may have a disproportionate influence on assemblage functioning in the experimental mixtures due to lack of sensitivity to osmotic shock.

Figure 10. Mixing experiment samples from (L to R) mangrove effluent (KW-15), 50:50 mixture (KW-15/KW-2) and the offshore end member (KW-2) during the August 2013 sampling.

During the third sampling (November 2013; no rainfall the week prior to sampling), high winds prevented small craft sampling of offshore open water stations so the No Name Key mangrove end member (KW-15, salinity = 42.6) was instead mixed with a channel end member sampled from the Old Wooden Bridge between No Name Key and Long Key (KW-16, salinity = 36). Though this sample was more likely to be impacted from the adjacent land mass than the offshore sample (4 mi south of Key West), it actually makes for a more likely scenario of what might happen during a storm-induced over wash of nearby No Name Key lagoon. It also turns out that bacterial production at KW-16 was similar to that offshore, KW-2 (14 (+/- 1.2) vs. 13 (+/-2.0) μg C L^{-1} d^{-1}, respectively), suggesting that it might not be as impacted by adjacent mangrove lagoons as initially suspected though the stations were sampled at different times of the year (August vs. November; Table 5, Figure 12). Effluent from No Name Key mangrove lagoon (KW-15) was mixed with adjacent channel water (KW-16) with increasing percentage (*i.e.,* 10, 20, 30, 40 and 50%).

Similarly to the August incubation of No Name Key water and open ocean water, mixing these end members enhanced bacterial production above that predicted by conservative mixing. This was the case both for incubations at T_0 and T_f (ca. 94 h later; Figure 12). At the experiment start (T_0), production increased from 10-25% (above predicted) with increasing percentage of mangrove water though this pattern was reversed to 38-31% by incubation end (T_f). This temporal reversal may reflect some parameter (*e.g.,* DO) that became limiting over incubation time in the fastest growing mixtures. Mixing had no affect on RDX mineralization but appears to have enhanced both HMX and phenanthrene mineralization (Figure 13). TNT mineralization

appeared to be enhanced above either end member in three of the five mixtures, as well, though there was no clear pattern with mixture percentage.

Table 5. Rates of bacterial production (incubation start, T_0; and end, T_f) and mineralization of TNT, phenanthrene (P), RDX and HMX (AVG (SD) µg C L^{-1} d^{-1}) for mixing experiment between mangrove effluent from near Fleming Key (KW-1) and offshore of Key West (KW-2; August 2013).

	Rate (AVG (SD) µg C L^{-1} d^{-1})					
	Bacterial Production		Mineralization			
Station	T_0	T_f	TNT	P	RDX	HMX
KW-15	186 (5.4)	102 (5.9)	0	1.21 (0.13)	2.04 (0.31)	2.57 (0.41)
Mix KW-15/2	158 (2.5)	7.3 (0.73)	0	1.63 (0.33)	0	1.9 (1.3)
KW-2	14 (1.2)	48 (2.9)	0.31 (0.13)	1.91 (0.26)	0.82 (0.57)	0

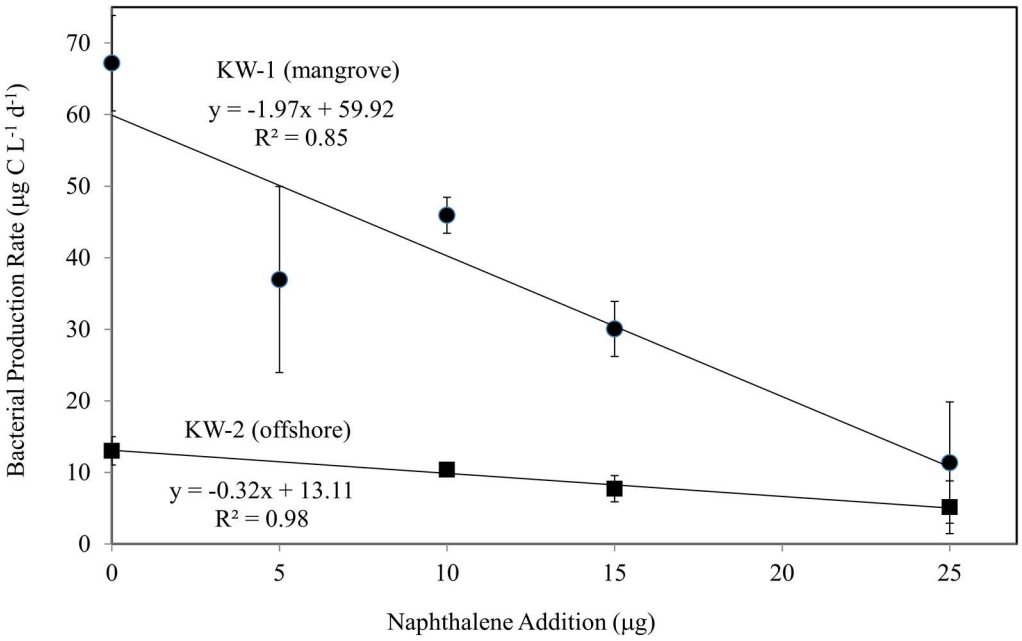


Figure 11. Organotolerance of bacterial assemblage as production (AVG (SD) µg C L^{-1} d^{-1}) decreases with naphthalene added (µg) to mixing experiment end members KW-1 (mangrove) and KW-2 (offshore; May 2013).

Some changes due to water mass mixing may be a simple function of the effect of different conditions on overall heterotrophic bacterial metabolism. However, when phenanthrene mineralization rate was normalized to bacterial production, this ratio was higher than predicted for each treatment and higher than each end member for all but the 50% mixture (which was no different from the 100% mangrove end member; Figure 14). This suggests that changes resulting from mixing end members preferentially enhanced aromatic contaminant degradation rates over those of noncontaminant natural organic matter, (*e.g.,* mangrove leachate) rather than simply stimulating mineralization by increasing overall heterotrophic carbon demand. This has important implications toward supporting the project hypothesis that frontal boundaries and mixing zones between water masses support aromatic contaminant degradation above that predicted from a simple survey of biodegradation rates along a salinity transect.

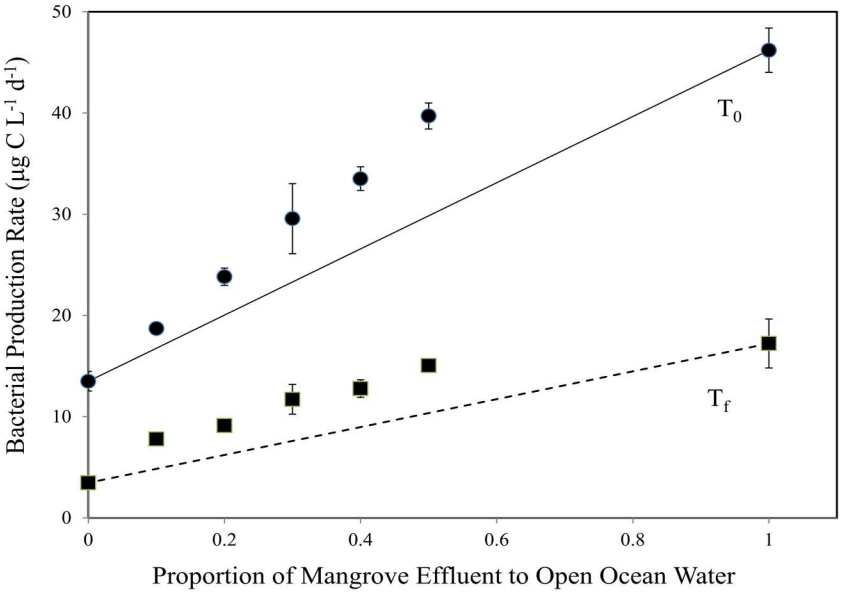


Figure 12. Bacterial production rate (AVG (SD) µg C L^{-1} d^{-1}) in mixing experiment with different proportions of No Name Key mangrove effluent (KW-15) and open ocean water from the adjacent waterway (KW-16; November 2013) at the incubation start (T_0) and after 95 h (T_f).

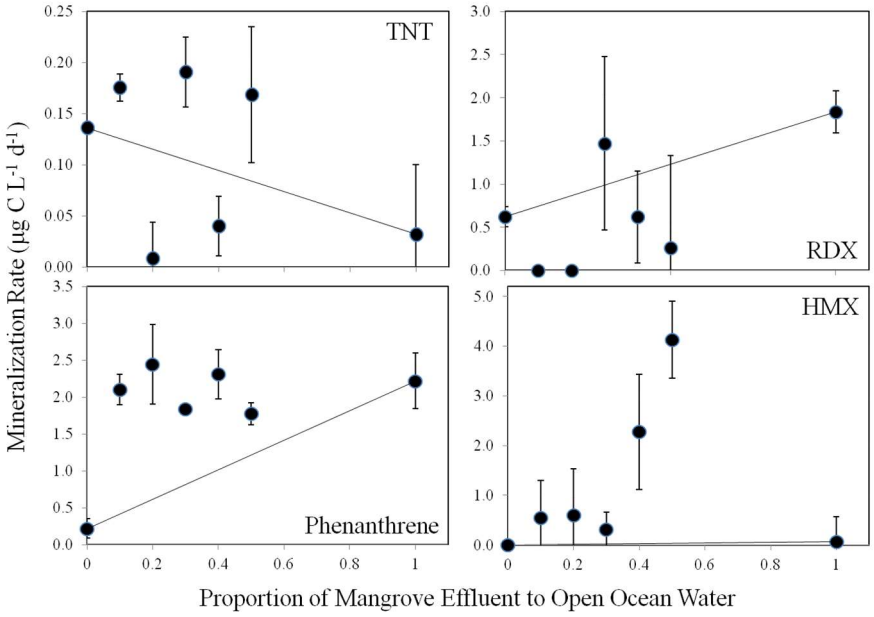


Figure 13. Mineralization of TNT, Phenanthrene, RDX and HMX (AVG (SD) µg C L^{-1} d^{-1}) in mixing experiment with different proportions of No Name Key mangrove effluent (KW-15) and open ocean water (KW-16; November 2013).

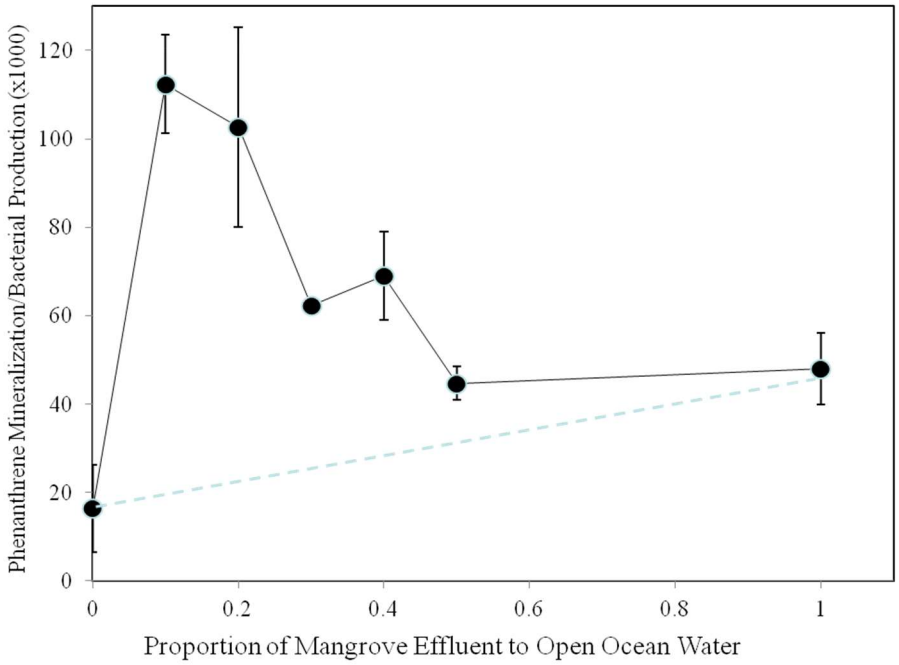


Figure 14. Phenanthrene mineralization/bacterial production rate (x1000) in mixing experiment with different proportions of No Name Key mangrove effluent (KW-15) and open ocean water (KW-16; November 2013).

The final Key West mixing experiment (October 2015) between No Name Key mangrove water (KW15) and the offshore end member (KW-41) was the only one of the Key West mixing experiments to show inhibition of both bacterial production and phenanthrene mineralization rates. Percentage inhibition increased with increasing concentration of mangrove end member water, 9-14% and 9-24% for production and phenanthrene mineralization, respectively (Table 6). These changes in the relative efficiency or carbon utilization in mangrove systems may not only be important with regards to anthropogenic contaminant attenuation but can also have larger scale climate change implications as these subtropical and tropical systems are important for carbon storage (Donato et al. 2011).

Another methodological experiment was performed first in this October 2015 sampling involving the timing and preparation of the mangrove water to the mineralization assay tubes. It is possible that preincubation of the isotope in the charged test tube (1 h prior to the commencement of the mineralization assay) would have some effect on rates. Humics present in the mangrove water could coat the isotopically labeled substrate molecules and reduce mineralization via steric hindrance or they could solubilize the substrate more rapidly off the test tube surface (Kalantary et al. 2013). The latter could increase mineralization by increasing the encounter rate between bacteria and the now-solubilized isotope. Also if the mangrove bacterial assemblage was most responsible for substrate biodegradation (relative to the open ocean assemblage), then increasing contact time between the relatively concentrated cells in the mangrove effluent and the the isotope might increase mineralization rate so filtered controls were added to differentiate between these two factors. There appeared to be some stimulation of mineralization with the 1% preincubation and the 5% filtered preincubation but it was not consistent among treatments. With the lesser additions of mangrove water (especially 0.1%), it

is possible that there was little direct contact between the water and the isotope because of the small volumes (2 uL) involved.

Table 6. Rates of bacterial production and mineralization of phenanthrene (P) (AVG (SD) µg C L^{-1} d^{-1}; October 2015) in mixing experiments using water from offshore of Key West, FL and a Mangrove swamp at No Name Key, FL, USA. Effect of preincubating the Mangrove addition with the isotope in the charged tube (filtered and unfiltered to remove the Mangrove bacterial assemblage) on mineralization rates was also measured. Percent inhibition or stimulation refers to standard mixing assay relative to that predicted from conservative averaging of end member values. N/A = Not Applicable

	Rate (AVG(SD) µg C L^{-1} d^{-1})		
Station	Bacterial Production	Mineralization P	Notes (salinity)
KW-41	7.1 (0.55)	0.074 (0.008)	Offshore (36)
0.1% KW-15/99.9% KW-41	6.4 (0.34)	0.067 (0.018)	0.1% Mangrove Mix
	N/A	0.062 (0.011)	0.1% Preincubated
	N/A	0.058 (0.004)	0.1% Preincubated; filtered
	9	9	% Inhibited/Stimulated
1% KW-15/99% KW-41	6.0 (0.28)	0.067 (0.012)	1% Mangrove Mix
	N/A	0.061 (0.001)	1% Preincubated
	N/A	0.081 (0.014)	1% Preincubated; filtered
	17	9	% Inhibited/Stimulated
5% KW-15/95% KW-41	6.3 (0.68)	0.055 (0.020)	5% Mangrove Mix
	N/A	0.070 (0.002)	5% Preincubated
	N/A	0.059 (0.011)	5% Preincubated; filtered
	19	24	% Inhibited/Stimulated
KW-15	20 (2.7)	0.050 (0.020)	No Name Key Mangrove Water (36)

Of the four samplings in Lower Outer Banks, NC, mixing experiments were performed during August and November 2014 and August 2015. Based on the findings from the mangrove lagoon/open ocean mixing experiments at Key West, greater dilutions of high organic lagoon or bog water were used in all subsequent mixtures. In August 2014 (high rainfall event), Cypress (pocosin) bog water (Newport, NC; NC-46, salinity = 0.06) was mixed with Atlantic Ocean water from about 2 miles outside of Beaufort Inlet past the Newport River frontal boundary (NC-40, salinity = 30; Figure 5). Mixtures included full strength seawater (0% fresh) with increasing amounts of freshwater (1, 2, 5, 10, 25%) up to full strength bog water (ca. 100% fresh). Although there was no discernible pattern amongst mixing amount and mineralization of RDX or HMX, both TNT and phenanthrene mineralization rates were highest at 2% freshwater addition to seawater (0.18+/-0.05 and 1.2+/-0.30 µg C L^{-1} d^{-1}, respectively; Table 7). The mixture also had the highest percentage of mineralization of two substrates relative to bacterial production (0.98 and 6.1, respectively) suggesting that enhanced biodegradation was not a simple function of overall increased bacterial metabolism (Table 7).

For November 2014 experiments (moderate rain event), mixes included 0, 1, 2, 5, and 100% Cypress bog water additions into seawater. HMX and RDX mineralization were most rapid at Cypress bog end member NC-66 (salinity = 0.11) and all mixes appeared to reduce mineralization below that of end members (NC-66; NC-67, Radio Island, salinity = 33; Table 8). Phenanthrene mineralization was lowest in the freshwater bog, highest in full strength seawater, and higher than predicted in mixes (based on conservative mixing of end members; Table 8). This was very similar to the pattern seen in August 2014 sampling though values were generally lower in November as might be expected with the generally lower temperatures. TNT

mineralization may have been highest in 1 and 2% freshwater additions to seawater though high error bars associated with the seawater end member (1.61 +/- 1.52 μg C L^{-1} d^{-1}) made direct comparison problematic (Table 8).

Table 7. Rates of bacterial production and mineralization of TNT, phenanthrene (P), RDX and HMX (AVG (SD) μg C L^{-1} d^{-1}; August 2014) and mineralization normalized to bacterial production (%) in mixing experiments with water from the Lower Outer Banks, NC, USA.

		Rate (AVG(SD) μg L^{-1} d^{-1})								
	Bacterial	Mineralization				Mineralization/Production (%)				
Station	Production	TNT	P	RDX	HMX	TNT	P	RDX	HMX	Notes
NC40	18 (0.78)	0	0.98 (0.12)	3.7 (1.7)	8.1 (3.3)	0	5.4	20	45	Marine (offshore)
NC41	18 (1.7)	0.16 (0.07)	0.76 (0.06)	2.1 (0.33)	4.4 (1.2)	0.92	4.3	12	25	1%Fresh/99%Marine
NC42	19 (0.69)	0.18 (0.05)	1.2 (0.30)	1.9 (0.52)	5.9 (1.4)	0.98	6.1	10	31	2%Fresh/98%Marine
NC43	20 (0.70)	0	0.82 (0.12)	3.4 (1.1)	7.4 (1.1)	0	4.2	18	38	5%Fresh/95%Marine
NC44	19 (1.4)	0	0.85 (0.23)	3.8 (0.74)	5.9 (0.72)	0	4.6	20	32	10%Fresh/90%Marine
NC45	16 (0.74)	0	0.88 (0.27)	2.5 (0.33)	5.0 (1.7)	0	5.6	16	32	25%Fresh/75%Marine
NC46	36 (3.4)	0	0.34 (0.31)	2.6 (0.35)	8.9 (2.5)	0	0.94	7	24	100%Fresh

Table 8. Rates of bacterial production and mineralization of TNT, phenanthrene (P), RDX and HMX (AVG (SD) μg C L^{-1} d^{-1}; November 2014) in mixing experiments with water from the Lower Outer Banks, NC, USA. BD = Below Detect

		Rate (AVG(SD) μg C L^{-1} d^{-1})				
	Bacterial	Mineralization				
Station	Production	TNT	P	RDX	HMX	Notes (salinity)
NC-67	15 (0.82)	1.6 (1.5)	0.24 (0.04)	7.0 (1.2)	6.3 (1.9)	100% Marine (33)
NC-68	16 (0.85)	0.71 (0.36)	0.17 (0.012)	4.5 (0.38)	5.2 (0.71)	1% Cypress/99% Marine
NC-69	15 (0.60)	0.67 (0.25)	0.17 (0.020)	4.9 (1.1)	3.3 (0.33)	2% Cypress/98% Marine
NC-70	15 (0.63)	0.39 (0.16)	0.17 (0.003)	5.5 (1.6)	BD	5% Cypress/95% Marine
NC-66	159 (13)	0.19 (0.04)	0.036 (0.004)	10.6 (0.56)	11.3 (1.0)	100% Cypress Bog (0.11)

In an attempt to determine the role of different end member bacterial assemblages in biodegradation, each end member assemblage was separately removed via filtration (0.22 μm nom. pore dia.) in a 1% freshwater/99% seawater mix (November 2014, Table 9). Two possibilities for the effect of mixing on biodegradation would be that some inhibitory compounds (*e.g.,* humics), concentrated in the Cypress bog water, are being diluted out by seawater and then aromatic or energetic mineralization by the bog water assemblage is enhanced. Alternatively, some micronutrient or organic carbon itself (*e.g.,* priming; Kuzyakov 2010, Bianchi 2011) in bog water could be stimulating marine assemblages to enhance biodegradation. For RDX, both treatments resulted in lower mineralization rates than predicted based on multiplying end member degradation rates by the dilution factor (*e.g.,* 1% x NC-66 or 99% x NC-67). Mixing rarely seemed to stimulate RDX mineralization, but this result is not unexpected especially if some factor was important for RDX metabolism (*e.g.,* low DO concentration; Smith et al. 2015). The filtration process could increase DO and thus reduce RDX mineralization in mixes relative to unfiltered end members.

For TNT, phenanthrene, and HMX, filtering out the Cypress bog microbial assemblage resulted in mineralization rates that were lower than predicted (38, 67, and 30% of predicted values, respectively; Table 9). Conversely, filtering out the seawater assemblage (99% of the mixture) and adding unfiltered Cypress bog water resulted in biodegradation rates that were 4-5 orders of

magnitude higher than predicted. These two findings suggest that Cypress bog water harbors the bacterial assemblage primarily responsible for biodegrading TNT, phenanthrene and HMX and that mineralization in these waters may either be inhibited by elevated concentration of some factor in bog water (*e.g.,* humics) or may be stimulated by presence of some factor or nutrient in full strength seawater. Note that the similarity among mixing experiments using mangrove lagoon and Cypress bog water was that both these end members had high aromatic organic concentration (see DOC section) but a dissimilarity was there wide range in salinity with the mangrove lagoon often being hypersaline (salinity > 40) and bog water close to fresh (salinity < 0.5). If there were some relationship between lignin biodegrading assemblages and TNT, phenanthrene, and HMX biodegradation, then it stands to reason that the Cypress bog water assemblage is more responsible for contaminant biodegradation than the open ocean water assemblage. The Cypress bog assemblages would be exposed to much higher concentration of terrestrial aromatics and may even harbor lignolytic fungi that are normally outcompeted by bacteria amongst marine and estuarine microbial assemblages (Benner et al. 1986).

Table 9. Cypress swamp bacterial assemblage appears to be more responsible for enhanced biodegradation of TNT, phenanthrene (P) and HMX (AVG (SD) µg C L^{-1} d^{-1}) based on differential removal of each assemblage in mixing experiments (November 2014; Lower Outer Banks, NC). Color indicates whether the values for mixtures are lower or higher than would be predicted based on end member value and proportion of end member assemblage in mixture.

		Rate (AVG(SD) µg C L^{-1} d^{-1})				
	Bacterial	Mineralization				
Station	Production	TNT	P	RDX	HMX	Notes
NC-66 (Cypress)	159 (13)	0.19 (0.04)	0.036 (0.004)	11 (0.56)	11 (1.0)	Cypress Swamp (Newport; salinity = 0.11)
NC-67 (marine)	15 (0.82)	1.6 (1.5)	0.24 (0.035)	7.0 (1.2)	6.3 (1.9)	Radio Is. (Marine; salinity = 33)
NC-71 (1% filtered)	15 (1.2)	0.60 (0.28)	0.16 (0.009)	3.6 (0.46)	1.9 (0.97)	1% Cypress DOM/99% Marine Assemblage
NC-71 predicted	15	1.6	0.23	7.0	6.3	0.99 x NC67
NC-71 % predicted	102	38	67	51	30	Mineralization values lower than predicted
NC-72 (99% filtered)	0.27 (0.14)	0.31	0.029 (0.009)	0.035 (1.0)	1.3	1% Cypress Assemblage/99% Marine DOM
NC-72 predicted	1.6	0.0019	0.0004	0.11	0.11	0.01 x NC66
NC-72 % predicted	17	16201	8106	33	1129	Mineralization values higher than predicted

For the final Lower Outer Banks, NC mixing experiment in August 2015, the effect of incubation time and preincubating (1 h) the Cypress bog water with radiolabeled substrate were also examined. Cypress bog water (0 PSU, NC-108) was mixed (0.1, 1, and 5%) with full strength seawater from off of Beaufort Inlet (36 PSU, NC-109). During this experiment, there was essentially no effect on TNT or phenanthrene mineralization relative to that expected from proportionate averaging of the end member rates (Table 10). Bacterial production was enhanced by 38 (+/- 11) % above that expected for the lowest bogwater addition (0.1%; NC-114) at T_0 just after mixing There was no measureable T_0 effect with the 1% addition and -12 (+/-10) % inhibition at 5% addition. By T_{90} (end of the mineralization incubation), the effect on bacterial production had reversed with the 5% addition being 245% higher than predicted and was the only one of the mixes or end members to be higher after T_{90} than at T_0 (Table 10). These results reinforce the earlier finding that the effect of the mixing on bacterial metabolism is time dependant (Qiu et al. 2016). This may be an important feature of frontal boundary bacterial metabolism that should be considered during future data collected during surveys where the residence time is not known.

Table 10. Rates of bacterial production and mineralization of TNT and phenanthrene (P), (AVG (SD) µg C L^{-1} d^{-1}; November 2014) in mixing experiments with water from the Lower Outer Banks, NC, USA. "pre" indicates samples where the Cypress bog amendment was preincubated with the charged tubes (1 h) prior to the mineralization assay. N/A = Not Applicable, BD = Below Detect.

	Rate (AVG(SD) µg C L^{-1} d^{-1})					
	Bacterial	Mineralization				
Station	Production	TNT	P	TNT pre	P pre	Notes (salinity)
NC-107	6.7 (0.7)	0.16 (0.10)	0.14 (0.01)	N/A	N/A	100% Marine (36)
NC-114	9.3 (0.3)	0.14 (0.02)	0.10 (0.01)	BD	0.18 (0.04)	0.1% Cypress/99.9% Marine
NC-113	7.4 (1.7)	0.16 (0.03)	0.10 (0.02)	0.12 (0.04)	0.19 (0.05)	1% Cypress/99% Marine
NC-112	8.7 (0.4)	0.14 (0.03)	0.10 (0.02)	BD	0.60 (0.42)	5% Cypress/95% Marine
NC-108	72 (3.1)	0.14 (0.01)	0.08 (0.03)	N/A	N/A	100% Cypress Bog (0.0)

Table 11. Rates of bacterial production (AVG (SD) µg C L^{-1} d^{-1}; November 2014) at the start (T_0) and finish (T_{90}; 90 h) of mixing experiments with water from the Lower Outer Banks, NC, USA.

	Rate (AVG(SD) µg C L^{-1} d^{-1})						
	Measured		Predicted		% of predicted		
Station	T_0	T_{90}	T_0	T_{90}	T_0	T_{90}	Notes (salinity)
NC-107	6.7 (0.7)	2.3 (0.18)	6.7	2.3	100 (10)	100 (8)	100% Marine (36)
NC-114	9.3 (0.3)	3.3 (0.37)	6.8	2.3	138 (3)	142 (11)	0.1% Cypress/99.9% Marine
NC-113	7.4 (1.7)	4.3 (0.32)	7.4	2.4	101 (23)	179 (7)	1% Cypress/99% Marine
NC-112	8.7 (0.4)	9.8 (0.98)	10	2.8	88 (4)	345 (10)	5% Cypress/95% Marine
NC-108	72 (3.1)	13 (0.42)	72	13	100 (4)	100 (3)	100% Cypress Bog (0)

During the September 2015 Corpus Christi, TX sampling, two mixing experiments were performed between the offshore seawater (CC-2; 31.95 PSU) and either the freshwater end member (Oso River, CC-1; 2.76 PSU) or hypersaline mangrove flat (CC-4; 41.86 PSU). Though there was no measurable effect on bacterial production with the 0.1 and 1% addition, but it was stimulated 130 and 124% for the 5% addition of Oso River and mangrove flat water, respectively (Table 12). Phenanthrene mineralization was stimulated between 126 to 175% in the all but the 5% Oso River mix. TNT mineralization was stimulated 200 and 207% in the 5% addition for Oso River and the mangrove flat, respectively (Table 12). The results for TNT mineralization followed the same pattern as that for overall heterotrophic bacterial production. It should be noted that with regards to phenanthrene mineralization, given the elevated presence of petroleum industry operations in Corpus Christi Bay relative to our other study sites, it would be expected that the bacterial assemblage may be more chronically exposed to phenanthrene flux than at other locations (Park et al. 2002; Duran and Cravo-Laurean 2016). No effect of preincubation with the radiolabel was seen.

A mixing experiment was performed using effluent draining a *Spartina alterniflora* marsh (Canary Creek) with water from the adjacent Delaware Bay at the creek mouth confluence with Roosevelt Inlet, Lewes, DE, USA (September 2014; Table 13). Unlike the mixing experiments performed to date between hypersaline lagoon water or freshwater, Canary Creek effluent was only slightly different in salinity (25 PSU) relative to the bay end member at Roosevelt Inlet (27 PSU). The mixing experiment involved whole water (*i.e.,* unfiltered) bacterial assemblages with

a relatively low amount of Canary Creek effluent (10%) diluted by a higher amount of bay water (90%). Bacterial production of this 4 day incubation was slightly lower than predicted (73%) based on end members values. However, mineralization was much higher than the predicted values for TNT (600%) and somewhat higher for phenanthrene (115%) and RDX (120%). HMX mineralization was slightly lower than predicted (93%; Table 13).

Table 12. Rates of bacterial production and mineralization of TNT and phenanthrene (P), (AVG (SD) µg C L^{-1} d^{-1}; September 2015) in mixing experiments with water from the Corpus Christi Bay System, TX, USA. Percent of predicted is based on the average of the end member measured values and the proportion of each end member in the mixed sample. BD = Below Detect, ND = Not Determined.

	Rate (AVG(SD) µg C L^{-1} d^{-1})						
	Bacterial Production		Mineralization				
Station	Measured	% of predicted	TNT (measured)	TNT (% of predicted)	P (measured)	P (% of predicted)	Notes (salinity)
CC-2	170 (8)	100	0.104 (0.104)	100	0.047 (0.005)	100	100% Aransas Pass (31.9)
0.1% CC-1	160 (6)	94	BD	ND	0.070 (0.007)	150	0.1% River/99.9% Marine
1% CC-1	178 (15)	105	0.125 (0.040)	121	0.064 (0.022)	136	1% River/99% Marine
5% CC-1	221 (13)	130	0.201 (0.013)	200	0.046 (0.006)	99	5% River/95% Marine
CC-1	168 (20)	100	0.038 (0.013)	100	0.033 (0.006)	100	100% Oso River (2.76)
CC-2	170 (8)	100	0.104 (0.104)	100	0.047 (0.005)	100	100% Aransas Pass (31.9)
0.1% CC-4	158 (11)	93	BD	ND	0.059 (0.008)	126	0.1% Flat/99.9% Marine
1% CC-4	159 (14)	93	0.081 (0.021)	78	0.082 (0.036)	175	1% Flat/99% Marine
5% CC-4	216 (23)	124	0.212 (0.045)	207	0.074 (0.025)	160	5% Flat/95% Marine
CC-4	250 (32)	100	0.079 (0.003)	100	0.040 (0.007)	100	100% Mangrove Flat (41.9)

Table 13. Mixing experiments rates of bacterial production and mineralization of TNT, phenanthrene (P), RDX and HMX (AVG (SD) µg C L^{-1} d^{-1}; September 2014) in a marsh creek adjacent to lower Delaware Bay, Lewes, DE, USA.

	Rate (AVG(SD) µg C L^{-1} d^{-1})					
		Mineralization				
Station	Bacterial Production	TNT	P	RDX	HMX	Notes
DE-1	17 (1.5)	0	0.38 (0.01)	3.1 (0.13)	5.7 (0.69)	Roosevelt Inlet confluence
DE-2	92 (6.0)	0.70 (0.09)	0.19 (0.10)	3.5 (0.36)	10 (1.4)	Canary Creek headwaters
10% DE-2/90% DE-1	18 (0.88	0.42 (0.38)	0.41 (0.10)	3.8 (0.78)	5.7 (1.2)	10% Canary Creek/90% Roosevelt Inlet
predicted	24.5	0.07	0.36	3.2	6.13	0.1 x DE-2 + 0.9 x DE-1
% predicted	73	600	115	120	93	Values higher or lower than predicted

To summarize the results of the nine mixing experiments, rates were often enhanced by mixing for bacterial production (5 of 9 experiments) and mineralization of TNT (3 of 8 experiments) and HMX were frequently enhanced (3 of 9 experiments; Table 14). Phenanthrene mineralization was often stimulated (8 of 9 experiments) while RDX mineralization was only stimulated in one of six experiments during which it was measured (Table 14). Addition of low molecular weight organics from the terrestrial end member may help stimulate biodegradation in mixed incubations (Derrien et al. 2014).

Table 14. Mixing experiment often stimulated rates of bacterial metabolism and contaminant catabolism above that which would be expected based on averaging on the end members. The end members typically involved mixing relatively low nutrient and organic offshore seawater with coastally sourced, high organic water.

Location	Sampling	Bacterial Production	TNT	Phenanthrene	RDX	HMX
Key West (mangrove)	MAY '13					
	AUG '13					
	NOV '13					
	OCT '15					
NC (Cypress bog)	AUG '14					
	NOV '14					
	AUG '15					
DE (*Spartina*)	SEP '14					
Corpus Christi (mangrove)	SEP '15					

Key (% enhancement)
<5
5-20
21-50
51-100
>100

Frontal Boundary Mixing experiments were performed because locating and sampling water mass boundaries in nature can be difficult as these areas can be spatially narrow, poorly-defined from above the water level, and transient. In addition, although a sample may have salinity or other geochemical parameter that is intermediate between two water mass end members, determining the time from mixing (or residence time) in the boundary area can require expensive and cumbersome isotopic analyses of earth metals or gases. However, during the August 2013 sampling, we came across a frontal boundary between two water masses (here designated west (KW-F1) and east (KW-F3)) that could be delineated by *Sargassum* collected at the sea surface (Figure 15).

Bacterial production was only slightly higher at the interfacial sample of a *Sargassum* front found about a mile offshore south of Key West and there were little differences in mineralization of TNT, phenanthrene and RDX (Table 15). However, HMX mineralization appears to be stimulated from below-detect on each side of the front (F1 and F3) to 0.43 (+/-0.13) $\mu g\ L^{-1}\ d^{-1}$ at the interfacial sample (KW-F2). *Sargassum* was collected and incubated separately from this survey and was found to enhance bacterial production by about an order of magnitude.

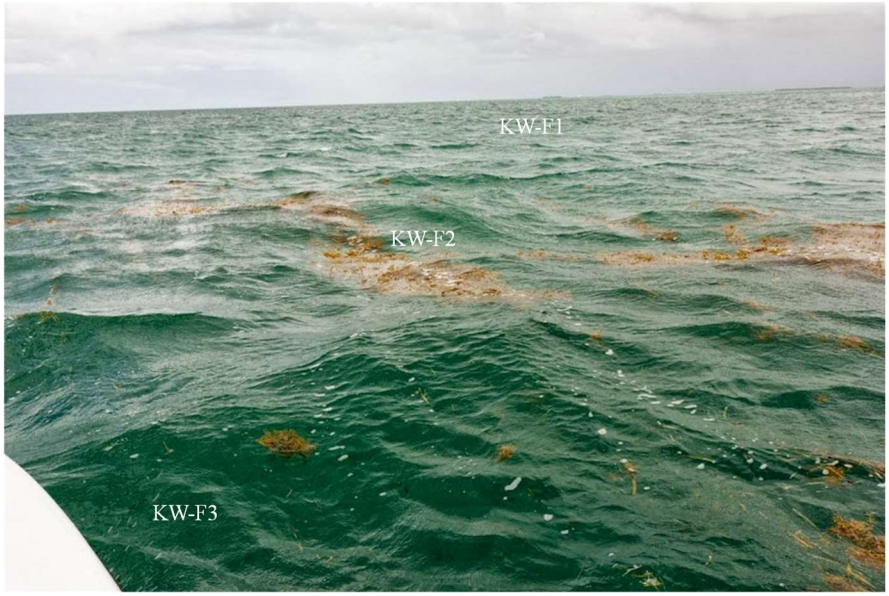


Figure 15. *Sargassum* collecting at frontal boundary between two water masses just south of Key West, FL. Surface samples were collected just west of the front (KW-F1), at the front (KW-F2) and just east of the front (KW-F3; August 2013).

Table 15. Frontal boundary rates of bacterial production and mineralization of TNT, phenanthrene (P), RDX and HMX (AVG (SD) µg C L^{-1} d^{-1}; August 2013) offshore of Key West, FL, USA. ND = Not Determined; BD = Below Detect

	Rate (AVG (SD) µg C L^{-1} d^{-1})					
	Bacterial	Mineralization				
Station	Production	TNT	P	RDX	HMX	Notes
KW-F1	13 (3.7)	7.8 (7.3)	2.25 (0.37)	0.78 (0.28)	0	West/ocean side
KW-F2	14 (1.0)	0.38 (0.35)	2.06 (0.59)	0	0.43 (0.13)	Interface
Sargassum	134 (3.6)	ND	ND	ND	ND	*Sargassum* incubation
KW-F3	13 (0.16)	0.59 (0.40)	1.91 (0.57)	0	0	East/bay side

During the 2014 sampling events in Lower Outer Banks, NC, transient estuarine fronts were encountered during all three cruises (April, Front-W; August, Front-NC-53; November, NC-55; Figure 5). Various frontal locations were likely reflective of the relative amount of rainfall during the week that preceded each sampling (April, low (0.87"); August, high (11"); November, moderate rainfall (1.5")). On 17 April 2014, a front formed near the mouth of Broad Creek in Bogue Sound (Front-W, Figure 5) which was characterized by large amounts of sea foam at the water mass confluence (Figure 16). Amount of rainfall the week prior to sampling was 0.87 inches, however, 0.64 inches fell in the area just prior to sampling of the front. The riverine side of the front (NC-W03, salinity = 27.6) had higher mineralization rates of phenanthrene and HMX, whereas, TNT mineralization was only detected on the sound side (NC-W01, salinity = 31.9; Table 16). The sea foam sample (NC-W0F1, salinity = 30), had no detectable mineralization rates for TNT, HMX, or phenanthrene.

The August 2014 sampling of Lower Outer Banks was preceded by 11 inches of rainfall the week prior and during the sampling. On 6 August 2014, runoff from this precipitation pushed a well-defined frontal plume offshore of Beaufort Inlet (Front NC-53, Figure 5). The inlet or

sound side of the front (NC-52, salinity = 27.5 PSU) was brown from Cypress bog input at the Newport River headwaters (inset, Figure 17). Surface water from the sound side of the front and at the interface itself had the highest ratio of mineralization to production for all four substrates (Table 17). Based on difference in salinity between frontal interface (29 PSU, NC-53) and open ocean sample (30 PSU, NC-51), the interface represented full strength seawater diluted by 3% with freshwater. Interestingly, the results showing enhancement of the ratio of TNT and phenanthrene mineralization to production matched up very well with those from the mixing experiment (1-2% dilution of full strength seawater with freshwater, Table 17). This is strong evidence that the mixing experiment can represent enhancement effects seen at frontal boundaries in nature. Because we know these effects to be time dependant (based on the change in bacterial production from T_0 and T_f), it also suggests that the time scales may be similar between the mixing experiment incubations (ca. 70 h) and that entrainment time for a water parcel at the estuarine front (*i.e.,* <70 h since mixing).

Table 16. Frontal boundary rates of bacterial production and mineralization of TNT, phenanthrene (P), RDX and HMX (AVG (SD) μg C L^{-1} d^{-1}; April 2014) in Bogue Sound, NC, USA. ND = Not Determined, BD = Below Detect.

		Rate (AVG(SD) μg C L^{-1} d^{-1})				
		Mineralization				
Station	Bacterial Production	TNT	P	RDX	HMX	Notes (salinity)
NC-W01	ND	0.63 (0.47)	0.31 (0.13)	ND	2.6 (1.5)	Bogue Sound Front (marine side, 31.9)
NC-W0F1	ND	BD	BD	ND	BD	Bogue Sound Front (interface, 30)
NC-W03	ND	BD	0.49 (0.13)	ND	4.6 (0.36)	Bogue Sound Front (river side, 27.6)

Table 17. Frontal boundary and salinity transect rates of bacterial production and mineralization of TNT, phenanthrene (P), RDX and HMX (μg C L^{-1} d^{-1}; August 2014) in Bogue Sound, NC, USA. BD = Below Detect.

		Rate (AVG(SD) μg C L^{-1} d^{-1})				
		Mineralization				
Station	Bacterial Production	TNT	P	RDX	HMX	Location (PSU)
NC-47	20 (2.2)	BD	0.22 (0.05)	3.1 (1.0)	14 (0.90)	Cypress Bog (0.01)
NC-46	36 (3.4)	BD	0.34 (0.31)	2.6 (0.35)	8.9 (2.5)	Cypress Bog (0.06)
NC-57	65 (1.6)	0.20 (0.04)	0.45 (0.04)	1.8 (0.91)	11.7 (6.1)	Newport River (0.7)
NC-56	81 (8.6)	BD	0.40 (0.18)	BD	8.7 (5.6)	Newport River (2.5)
NC-55	59 (4.9)	BD	0.66 (0.21)	0.86 (0.55)	4.8 (0.93)	Newport River (11.5)
NC-54	51 (4.1)	0.16 (0.03)	0.55 (0.27)	5.0 (1.5)	8.3 (1.6)	Newport River (21)
NC-52	34 (1.8)	BD	0.83 (0.50)	5.1 (1.1)	2.8 (2.4)	Sound side of front (27.5)
NC-53	22 (0.90)	0.12 (0.05)	0.72 (0.15)	2.9 (0.40)	6.5 (2.2)	Offshore side of front (29)
NC-40	18 (0.78)	BD	0.98 (0.12)	3.7 (1.7)	8.1 (3.3)	Offshore (30)
NC-51	21 (0.47)	0.16 (0.01)	BD	BD	BD	Offshore (30)

Figure 16. Frontal boundary in Bogue Sound, NC, USA (near former Navy MRP site at Cat Island) sampled 17 April 2014 for stations NC-W01 (left/riverside of front), NC-W0F1 (at interface/foam), and NC-W03 (right/bayside of front).

Figure 17. Mixing experiments and a frontal boundary survey were performed after a major rainfall event in the Lower Outer Banks, NC, USA (August 2014). This front separated Newport River Cypress bog effluent (left, NC-52) from open ocean water (right, NC-53).

During the November 2014 sampling, a salinity transect of the Newport River was performed (salinity ranged 1.02-27.4) and included a frontal boundary characterized by entrainment of gelatinous macrozooplankton (*i.e.,* ctenophore jellyfish) at the interface (Figure 18). TNT mineralization rate ranged from 0.14-0.45 μg C L^{-1} d^{-1} along this transect with the highest rates occurring at the frontal interface (0.30 +/- 0.091 μg C L^{-1} d^{-1}, NC-79) and at the station just to the riverine side of the front (0.45 +/- 0.17 μg C L^{-1} d^{-1}, NC-75; Table 18). In addition, phenanthrene mineralization rate ranged from 0.068-0.21 μg C L^{-1} d^{-1}, with highest rates at the front (0.17 +/- 0.074 μg C L^{-1} d^{-1}, NC-79) and at stations ocean side of the front (0.21 +/- 0.015 μg C L^{-1} d^{-1}, NC-77). When handling water samples in the lab, they appeared to be more viscous than those from the rest of the survey which is likely due to polysaccharide exudates associated with the entrained ctenophores (Pitt et al. 2009). In addition, the DOM production and its biodegradation can provide a nutrient pulse to the local environment (Tinta et al. 2010). Over the course of the three day incubation using the survey samples, bacterial production decreased by >50% in all but the frontal boundary sample incubation which decreased only 29% (NC-79: 18 +/- 1.9 μg C L^{-1} d^{-1}, T_0; 12.7 +/- 1.2 μg C L^{-1} d^{-1}, T_f; Table 18). This support of production over the incubation period may be the result of DOM and nutrient input from gelatinous macrozooplankton as has been reported by others for similar experiments (two day incubations; Blanchet et al. 2014). Despite this unique type of front, the findings of enhanced biodegradation were consistent with the working hypothesis of the program.

Figure 18. Pocosin bog effluent evident in prop wash (left); Newport River frontal boundary (middle); viscous and foamy water sample from boundary interface (right) which had a high density of gelatinous macrozooplankton (November 2014).

During the August 2015 sampling, a distinct turbidity front was encountered in the tidal creeks of western Bogue Sound near the confluence with the White Oak River. The particulate material on the turbid side of the front may have been resuspended by strong winds across an intertidal marsh flat in Bear Island Area Outstanding Resource (Figure 19). We were able to collect discreet samples from both the turbid tidal creek (NC-125; PSU = 29.5), relatively clear White Oak River/Bogue Sound seawater (NC-126; PSU = 31.4), as well as a sample at the interface between water masses (NC-127; PSU = 29.8) because of exceptionally calm conditions (Figure 20). Based on the salinity difference amongst the three samples, NC-127 was about 85% tidal creek water (NC-125). Bacterial production was about twice as rapid as that which would be expected based on averaging the values for each side of the front (in proportion to conservative mixing, e.g. 85% of NC-125 value plus 15% of that for NC-126). Phenanthrene mineralization was almost four times higher than expected based on conservative mixing (Figure 21). TNT mineralization rates were below detect in all three samples. Although both measures of heterotrophic carbon metabolism were enhanced at the boundary interface, phenanthrene

metabolism was disproportionately more rapid. One possibility for enhanced bacterial metabolism and degradation rates at frontal boundaries is the nutrient stimulation afforded by the recruitment and grazing activity of copepod zooplankters to these turbidity fronts (Deriso et al. 2014).

Table 18. Frontal boundary and salinity transect (NC-73-79; 15 November 2014) rates of bacterial production and mineralization of TNT, phenanthrene (P), RDX and HMX (AVG (SD) µg C L^{-1} d^{-1}) in Newport River of Lower Outer Banks, NC, USA. ND = Not Determined; BD = Below Detect.

	Rate (AVG (SD) µg C L^{-1} d^{-1})						
	Bacterial Production		Mineralization				
Station	**T_0**	**T_{3d}**	**TNT**	**P**	**RDX**	**HMX**	**Location (PSU)**
NC-66	159 (13)	33 (1.2)	0.19 (0.04)	0.036 (0.004)	10.6 (0.56)	11.3 (1.0)	Cypress Bog (0.11)
NC-73	17 (0.61)	8.6 (1.2)	0.14 (0.09)	0.17 (0.037)	5.3 (0.78)	4.0 (1.4)	Newport River (1.02)
NC-81	19 (1.0)	8.6 (1.5)	0.15 0.07)	0.17 (0.093)	BD	BD	Newport River (5.6)
NC-74	18 (0.35)	8.6 (0.62)	0.22 (0.06)	0.068 (0.026)	3.1 (1.6)	4.3 (0.84)	Newport River (11.08)
NC-80	21 (1.7)	7.3 (0.63)	0.28 (0.18)	0.15 (0.07)	4.4 (1.7)	3.4 (2.0)	Newport River (18.02)
NC-75	20 (0.77)	ND	0.45 (0.17)	0.10 (0.01)	BD	2.9 (1.0)	Newport River (20.25)
NC-79	18 (1.9)	12.7 (1.2)	0.30 (0.09)	0.17 (0.07)	BD	BD	Front (ctenophore; 25.5)
NC-77	19 (2.7)	ND	0.14 (0.05)	0.21 (0.02)	BD	11 (2.3)	Newport River (27.4)
NC-67	15 (0.82)	6.3 (0.27)	1.6 (1.5)	0.24 (0.04)	7.0 (1.2)	6.3 (1.9)	Radio Is. (marine; 33)

Figure 19. Turbidity front located near the confluence of Bogue Sound and the White Oak River, NC, USA (August 2015). Particulate and high DOC on the turbid side of the frontal interface seems to result from wind-driven resuspension of benthic material across an intertidal flat in the Bear Island Area Outstanding Resource draining into a marsh creek (Google Maps).

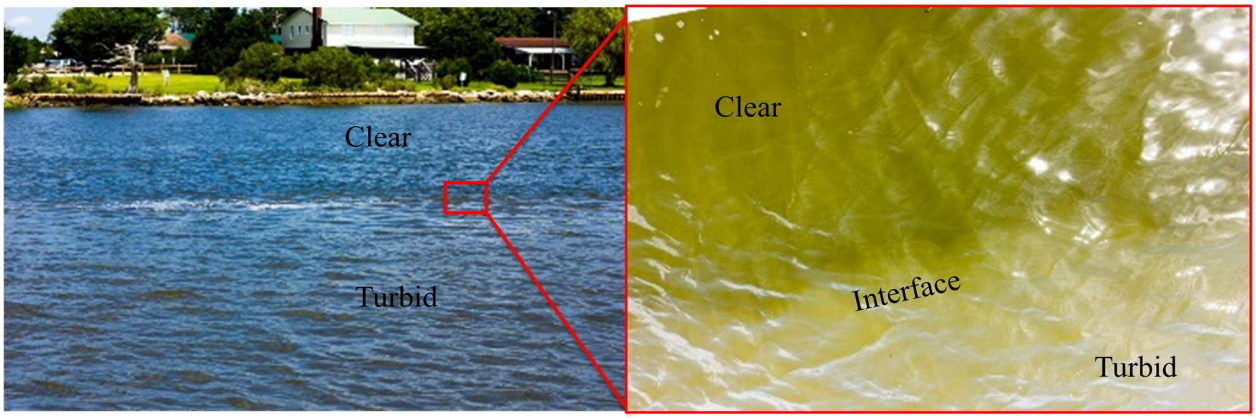


Figure 20. Frontal boundary adjacent to Bear Island Area Outstanding Resource (August 2015). Front Clear seawater from the White Oak River (NC-126; PSU = 31.4) was sampled along with turbid water from a tidal marsh creek (NC-125; PSU = 29.5) and the interface (NC-127; PSU = 29.8) (Google Maps).

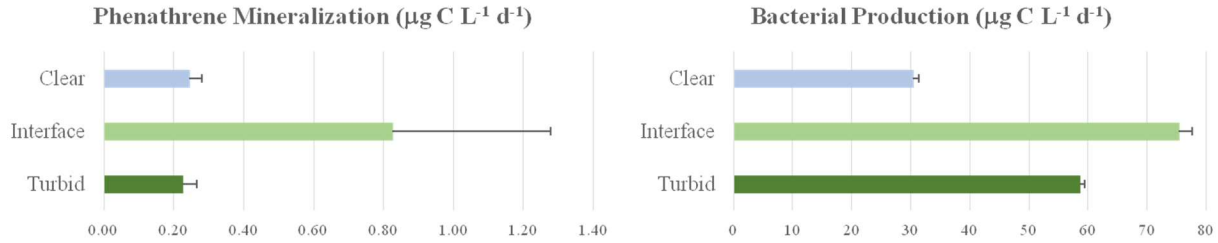


Figure 21. Turbidity front located near the confluence of Bogue Sound and the White Oak River, NC, USA (August 2015). Both rates of bacterial production and phenanthrene mineralization (µg C L^{-1} d^{-1}) were both enhanced in the sample collected from the interface between the two water masses.

During coastal estuarine surveys as part of ER2123, the seed project, as well as the preceding ER1431 that provided the initial observation, we found that bacterial metabolism was often enhanced at frontal boundaries (Table 19). We also established the time dependent effect of mixing on bacterial growth. Even though we do not know the residence time of samples collected at the front interface, the results of the surveys often correlated very well with the laboratory mixing experiments using field collected end members. Together these data provide strong evidence that frontal interfaces create previously undescribed enhancement of naturally attenuating processes for anthropogenic contaminants.

DOC Analyses DOC concentrations for sites around Key West ranged from 93 to 251 µM and had a weak positive correlation with salinity (R^2 = 0.23; P<0.05). This appeared to be due to evaporation of shallow open water on the shelf rather than seawater diluted with terrestrial-rich freshwater as corresponding salinities ranged only from 36.01 (KW-13/14) to 36.74 (KW-5). DOC was correlated to absorption at 254 nm (a_{254}; R^2 = 0.76, P<0.05). Specific ultraviolet absorption (SUVA) which is correlated to aromatic ring content in organic matter ranged from 0.51 to 2.13. These values are characteristic of marine or planktonic dominated systems with some terrestrial inputs (Weishaar et al. 2003).

Carbon stable isotope (δ^{13}C-DOC) values indicated largely phytoplankton-derived, marine DOM in coastal waters of Key West, with some notable terrestrial input. Offshore values typically

ranged from -19 to -22‰, typical for coastal seawater DOC. Closer inshore samples (*e.g.,* KW-4, Channel Key mangrove) had relatively depleted values of -25.16‰ reflecting terrestrial input.

Table 19. Similar to the findings from the mixing experiments, samples from interfaces sampled from coastal estuarine and marine fronts often showed elevated rates of bacterial metabolism and phenanthrene catabolism (µg C L^{-1} d^{-1}) relative to the averaging of the values from each side of the two water masses.

Location	Sampling	Bacterial Production	TNT	Phenanthrene	RDX	HMX	Notes	Key (% enhanced)
Kahana Bay	AUG '06						Riverine	<5
Charleston	JUN '11						Tidal	5-20
Key West	MAY 13						Sargassum	21-50
North Carolina	AUG '14						Offshore/rain	51-100
	NOV '14						Ctenophore	>100
	AUG '15						Turbidity	

DOM EEM fluorescence supported the DOC findings (Figure 22). The offshore sample was measured in the frontal zone (KW-F1) encountered in August 2013. That pattern was very similar to the mangrove water sampled at No Name Key. By contrast, fresh *Sargassum* DOM, leached overnight into seawater, produced a very different EEM pattern, unlike that measured on the majority of our samples and indicated by the offshore frontal zone station, KW-F1. Also of note, the magnitude of fluorescence for No Name Key mangrove water was nearly 40-fold greater than the Offshore sample. The latter also had stronger protein fluorescence (centered on Ex/Em 280/340), which reflected the marine phytoplankton signature in the coastal seawater DOM surrounding Key West. Based on this EEM analysis of unknown organic matter sources in a water mass, we would be able to differentiate between mangrove effluent, *Sargassum*, terrestrial and open ocean DOM signatures in coastal seawater surrounding a wet, tropical ecosystem such as Key West or Veiques, Puerto Rico.

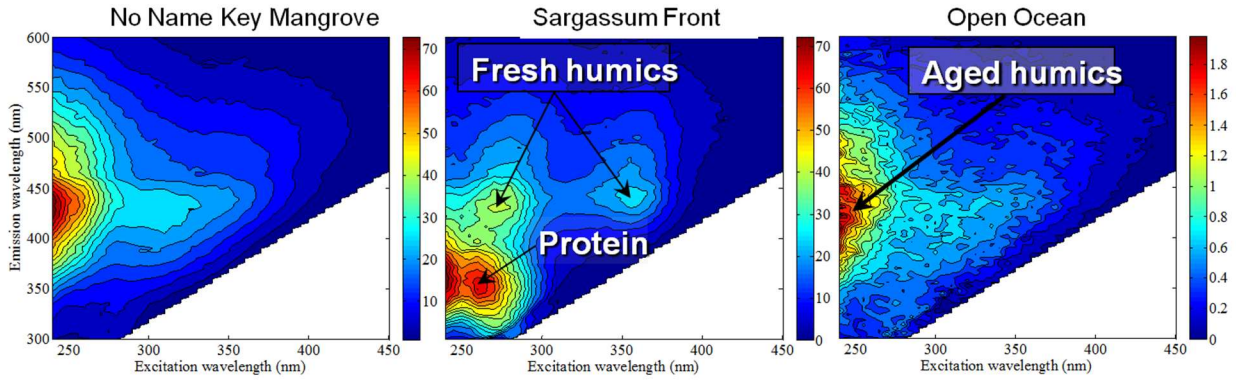


Figure 22. EEM fluorescence analyses of potential OM sources in water samples from Key West during 2013 samplings.

For sampling around Lower Outer Banks, DOC concentrations ranged from 1.33 to 36.89 mg L^{-1} (111 to 3073 µM) and had a strong negative correlation with salinity for both New (R^2 = 0.9885; P<0.05) and Newport Rivers (R^2 = 0.9305; P<0.05). This appears to be an effect of diluting terrestrial-rich freshwater (*e.g.,* Cypress bog in Newport River) with seawater (Figure 23) though there could be some production of DOC at salinity of 11.5 in Newport River (August, NC-55) which was at a frontal boundary (Figure 5).

DOM excitation-emission (EEM) fluorescence supported the DOC findings for North Carolina waters (Figure 24). EEM fluorescence in the Cypress swamp was 20-fold greater than in the coastal open ocean, reflecting the dilution seen in DOC across the salinity gradient (Figure 20). The Ctenophore front EEM was more similar to the open ocean yet shared features with the Cypress Swamp. These features can be seen in the fluorescence signal at 240 nm excitation and 475-525 nm emission. The signal is noticeable in the Ctenophore front but noticeably lacking in open ocean. Generally, terrestrial humic and fulvic fluorescence is >400 nm owing to extensive conjugation of humic substances derived from plant material, such as lignin. Fluorescence "blue shifts" towards lower wavelengths as conjugation is disrupted via degradation. This disruption is observed as loss of fluorescence emission from 475-525 nm from the Cypress swamp to Ctenophore front and lack of this signal in the open ocean EEM (Figure 24). Note that the core aromatic fluorescence remains in the coastal open ocean EEM though dramatically reduced as a function of both dilution and degradation. This analysis could be used to track shoreside input (*e.g.,* from a range) into coastal or open ocean seawater.

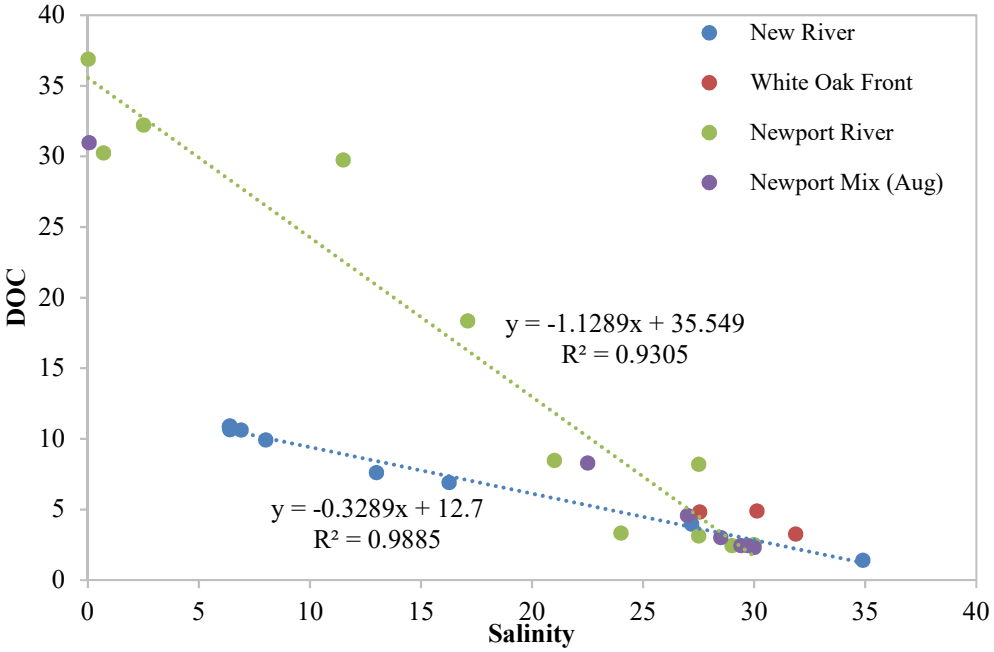


Figure 23. Relationship between DOC and salinity for Lower Outer Banks, NC, water bodies. Carbon stable isotope (δ^{13}C-DOC) for April 2014 surveys ranged from -25.27‰ (NC-03) to -27.55‰ (NC-F2) and for August 2014 ranged from -24.64‰ (NC-02) to -29.11‰ (NC-52). These values generally reflect the large terrestrial input for the Lower Outer Banks systems. Primarily this terrestrial input occurs from outwelling of pocosins (Cypress swamp). Little evidence from stable isotopes suggests that salt marshes export DOC (salt marsh δ^{13}C-DOC values are typically >20‰). The one outlier was the Cat Island (NC-01) station that was -37.29‰ during August and may have reflected some methanotrophic source of DOC.

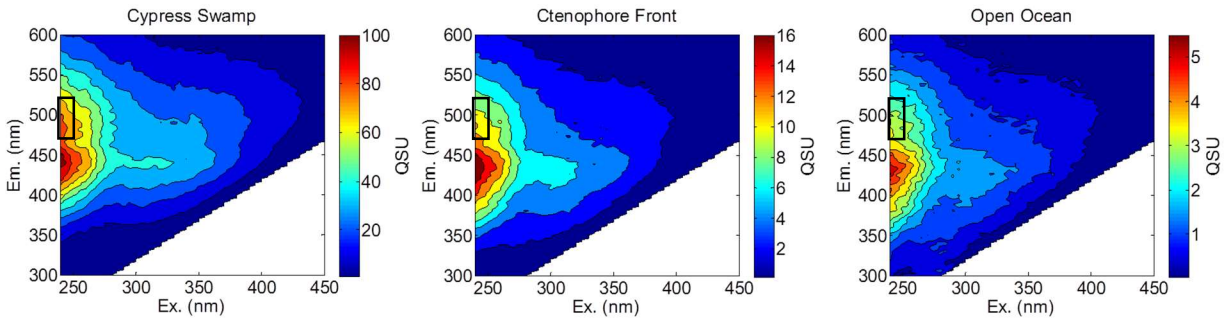


Figure 24. EEM fluorescence analyses of potential OM sources in water samples from Lower Outer Banks, NC, USA during CY14 samplings. Black boxes indicate fluorescence region attributed to terrestrial humics exported by the Cypress Swamp.

Lignin concentration correlated with a254 (Figure 25) and decreased somewhat conservatively with salinity (Figure 26). There appears to be some relationship between RDX and HMX mineralization rate (µg C L^{-1} d^{-1}) and absorbance at 245 nm (a254, 1/m) which is a surrogate for dissolved lignin concentration though this may be dependent upon type of assemblage (freshwater vs. estuarine) for Lower Outer Banks, NC, stations during three samplings in 2014 (Figure 27). There may be some threshold of lignin (or more likely associated humics) that inhibits bacterial production (Figure 28).

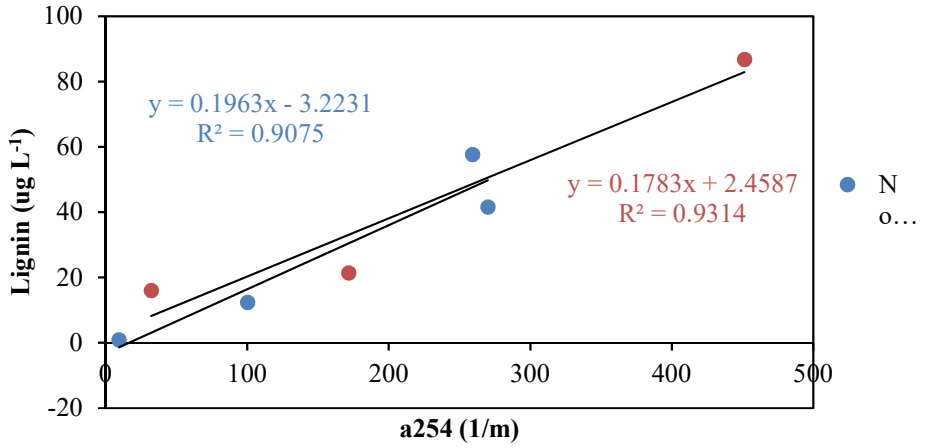


Figure 25. Relationship between lignin concentration and absorbance at 254 nm in August and November 2014 for Lower Outer Banks, NC, water bodies.

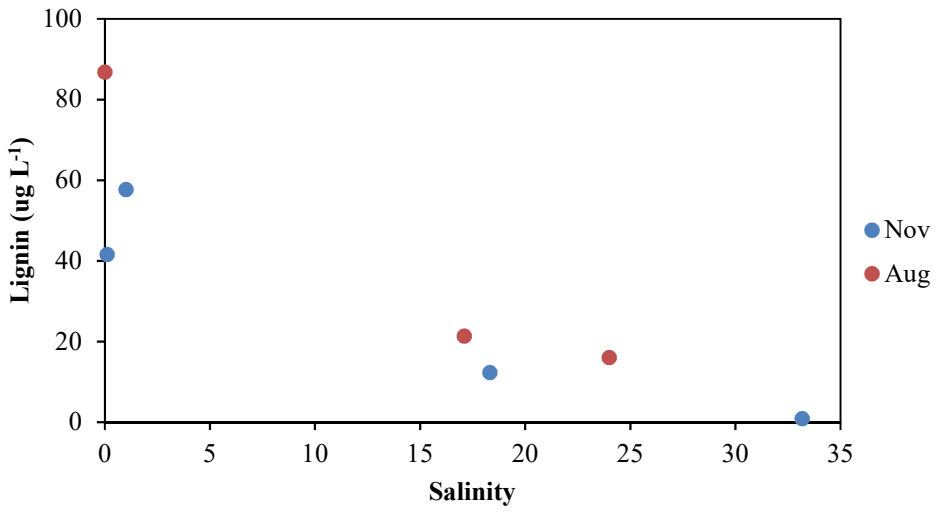


Figure 26. Relationship between lignin concentration and salinity in August and November 2014 for Lower Outer Banks, NC, water bodies.

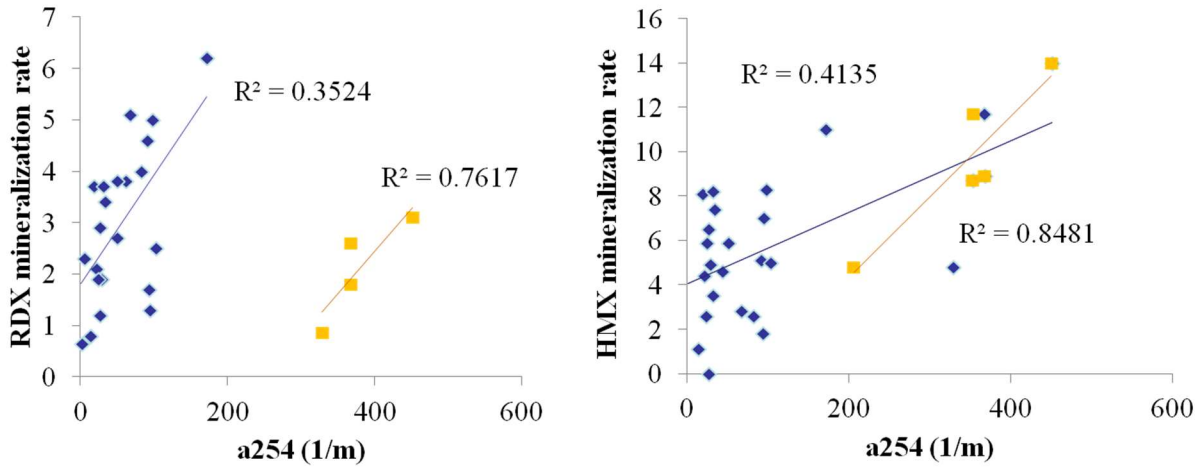


Figure 27. There appears to be some relationship between RDX and HMX mineralization rate (µg C L^{-1} d^{-1}) and absorbance at 245 nm (a254, 1/m) which is a surrogate for dissolved lignin concentration though this may be dependent upon type of assemblage (freshwater vs. estuarine) for Lower Outer Banks, NC, stations during three samplings in 2014.

Aromatic concentrations in DOM and POM Absorption at 254 nm is used in this project as a proxy for aromatic carbon, which is hypothesized to sustain microbial communities able to degrade nitrogenous aromatic such as TNT and other explosives, as well as PAHs. In Y3, the environments studied show a great diversity with respect to aromatic DOM and POM loads (Figures 29, 30). Two trends are of note from the comparison of subtropical (North Carolina) to humid sub-tropical (Corpus Christi) and to tropical savanna (KW) climate regimes. In the sub tropical climates of Corpus Christi and Key West, aromatic DOM was largely concentrated in isolated mangrove wetlands (Key West) and salt flats (Corpus Christi) (Figure 29A). In contrast, extensive wetland development around coastal North Carolina (pocosins draining into salt marsh) contributes roughly 3x higher concentrations of aromatic DOM as evidenced by the

Newport River. The Newport River exhibited generally conservative mixing at salinities of 5-36, extending out through Beaufort Inlet to the coastal Atlantic Ocean (Onslow Bay) (Figure 29B). At lower salinity (<3) a substantial decrease in a254 (ca. 50 m^{-1}) suggested a loss of the aromatic DOM in this estuarine system. It is possible that an estuarine turbidity maximum (ETM) developed but was not observed during the sampling of this system. ETM are sites of potential flocculation and aggregation of organic matter due to changes in ionic strength which depress electrostatic repulsion of freshwater colloidal material. Moreover, this loss could be due to biological activity in the oligohaline reach of this estuary via the release of exopolymeric substances (EPS) that can serve as "glue" binding up larger organic compounds. Future work could investigate the geochemistry of surface sediments in this system and the potential for entrainment of aromatic organic matter in the surface sediments and nepheloid layer.

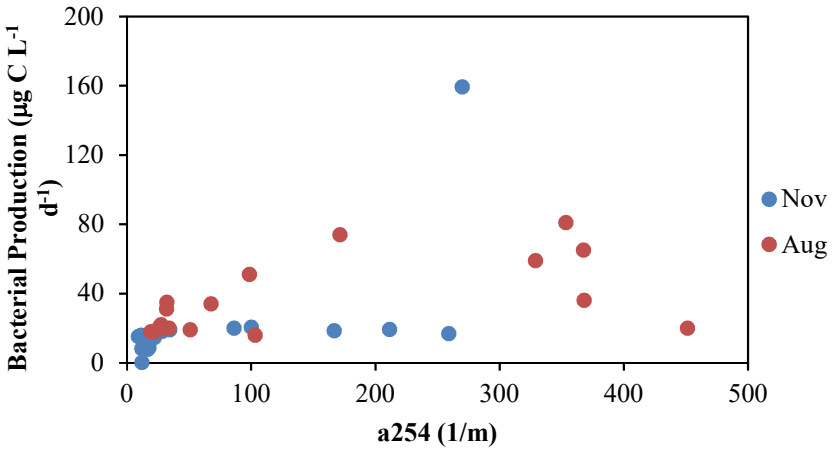


Figure 28. Relationship between bacterial production and lignin concentration in August and November 2014 for Lower Outer Banks, NC, water bodies.

Sites in Bogue Sound and in the White Oak River also show fairly conservative mixing with salinity (Figure 29B). The White Oak River was sampled at a frontal boundary related to the outwelling from a salt marsh creek which flows into the river proper. The aromatic concentrations decrease in a typically conservative manner across the frontal boundary. However, evidence of enhance bacterial production and aromatic C mineralization across this frontal zone suggests that concentration-independent processes enhance the degradation of aromatic organic matter.

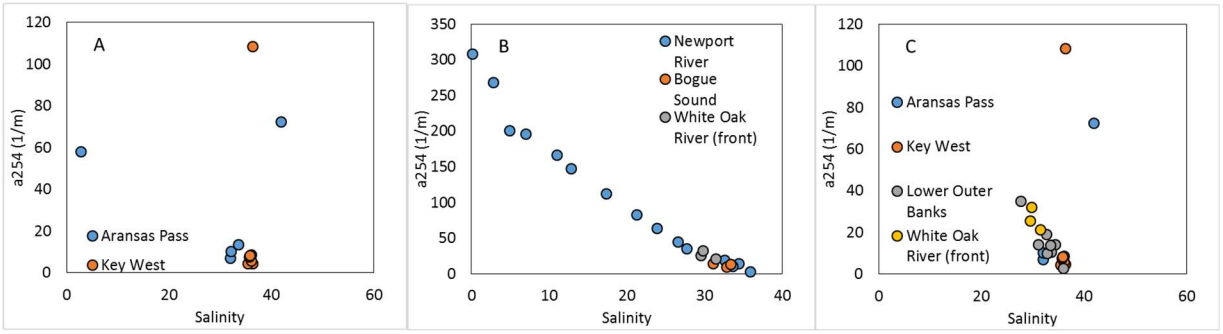


Figure 29. CDOM absorption showing aromatic organic carbon in particles in (A) Aransas Pass, TX and Key West, FL; (B) Newport River, Bogue Sound, and White Oak River in the Lower Outer Banks, NC; (C) TX, FL, and NC results at high salinity showing the effects of evapoconcentration on enriching aromatic material in the mangroves of TX (Aransas Pass) and FL (Key West).

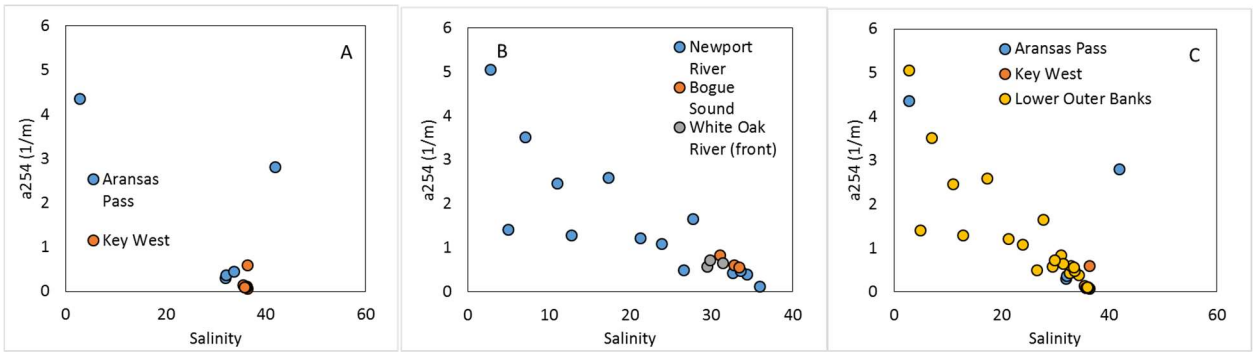


Figure 30. BEPOM absorption showing aromatic organic carbon in particles in Aransas Pass, TX; Key West, FL; and Lower Outer Banks, NC.

Aromatic organic matter can be present in particulates in addition to dissolved materials. Base-extracted particulate organic matter (BEPOM) was developed as part of past collaborative research and provides a complimentary insight to aromatic C in POM (Figure 30). First, POM carries far lower amounts of aromatic C than does DOM, as indicated by a254 values which were ca. 20-60 times less than DOM across all climate regimes. Both Corpus Christi and Key West BEPOM values from open water samples were <1 m^{-1} (Figure 30A). Also of note, the Key West mangrove lagoon water was nearly absent in particles. However, the fresher urbanized Oso River had a higher aromatic C content in POM than did the salt flat adjacent to Aransas Pass which had salinity >40. This likely was due to the lower energy environment of the salt flat and the mangrove lagoon (KW) which would facilitate particle deposition rather than resuspension. By contrast, the Newport River showed an aromatic POM content similar to Oso River, both fluvial environments in which particles should remain in suspension (Figure 30B). In general, a254 values in BEPOM of the Newport River decreased with salinity though the relationship was not as strong as for DOM. Aromatic POM was more similar in terms of concentration across the climatic gradient studied (Figure 30C). The range of values generally decreased as a function of salinity save for the salt flat mangrove sample from Corpus Christi. A first conclusion from this study is that aromatic C flux in coastal waters appears to be dominated by the DOM pool.

Aromatic C as measured by CDOM absorption at 254 nm is strongly correlated to DOC concentration in the Newport River estuary in 2014 (Figure 31). This relationship holds despite the different hydrodynamic conditions between extreme freshwater discharge during August and normal conditions in November. Our analysis is important to establish the utility of CDOM as a tracer for aromatic organic matter. Future work will continue on refining this model broadly across multiple coastal environments situated across climatic regimes.

Sources of aromatics in DOM and POM elucidated by EEM-PARAFAC EEM fluorescence of CDOM and BEPOM was measured and modeled with parallel factor analysis (PARAFAC). A five component model was validated (Figure 4) and each component was matched to the OpenFluor database and several matches for each component were determined. This database allows for a consensus identity to be established for the PARAFAC components. Component 1 (C1) was identified as soil derived organic matter. C2 was identified as protein-like fluorescence. C3 was identified as microbial humic substances, which is generated as bacteria degrade and modify organic matter. C4 was identified as quinone-like fluorescence and is

thought to correspond to enhanced heterotrophic activity. C5 is a residual terrestrial fluorescence signal of DOM that escapes estuaries and coastal waters and is transported well offshore.

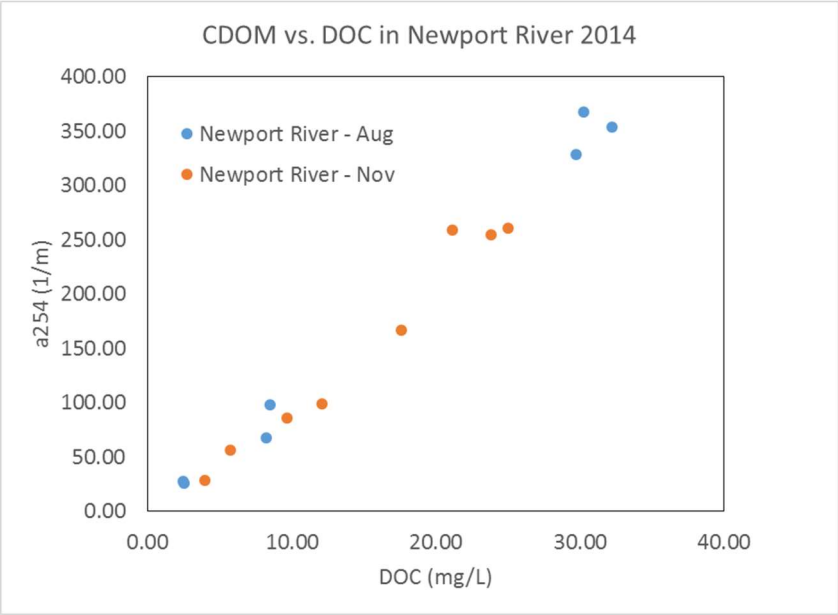


Figure 31. Relationship used to predict DOC concentration from a254 in the Newport River estuary.

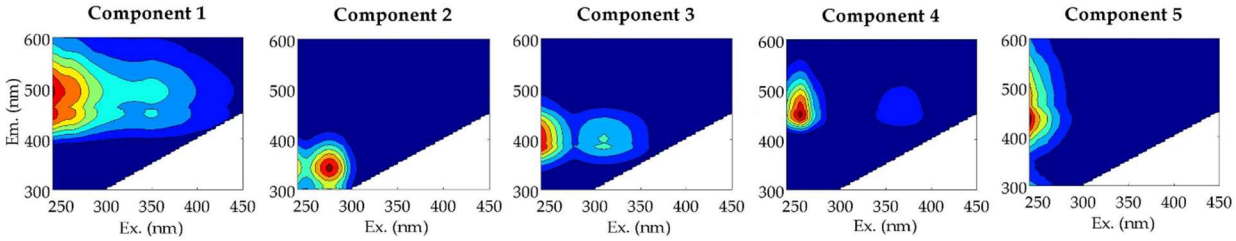


Figure 32. EEM-PARAFAC results showing five components that characterize CDOM and BEPOM in this study.

Utilizing this scheme of fluorescence markers, characterization of DOM and POM in the three coastal environments studied for this project was done (Figure 33). In general, NC estuaries were enhanced in terrestrial organic matter owing to the large a254 values and comparatively large contributions of soil derived and terrestrial humic material (Figure 33). Aransas Pass OM was a more equal mixture of humic and protein-like material with a clear dominance by microbial processing (C3; Figure 33). Key West exhibited a pattern intermediate between North Carolina and Corpus Christi. Examining the organic matter pools separately elucidated key differences in the source of aromatic OM.

CDOM was dominated by soil derived material and by microbial humic material (C1 and C3 respectively, Figure 33). North Carolina had the largest fluorescence overall but it is worth noting that both Corpus Christi and Key West exhibited very strong contributions by C3. This indicates that each of these environments have extensive processing of OM brought into them. Moreover, C4 was as important as C5 which suggested that despite the aromatic nature of the OM (strong C1, C3, and C5 signals) extensive microbial activity was present.

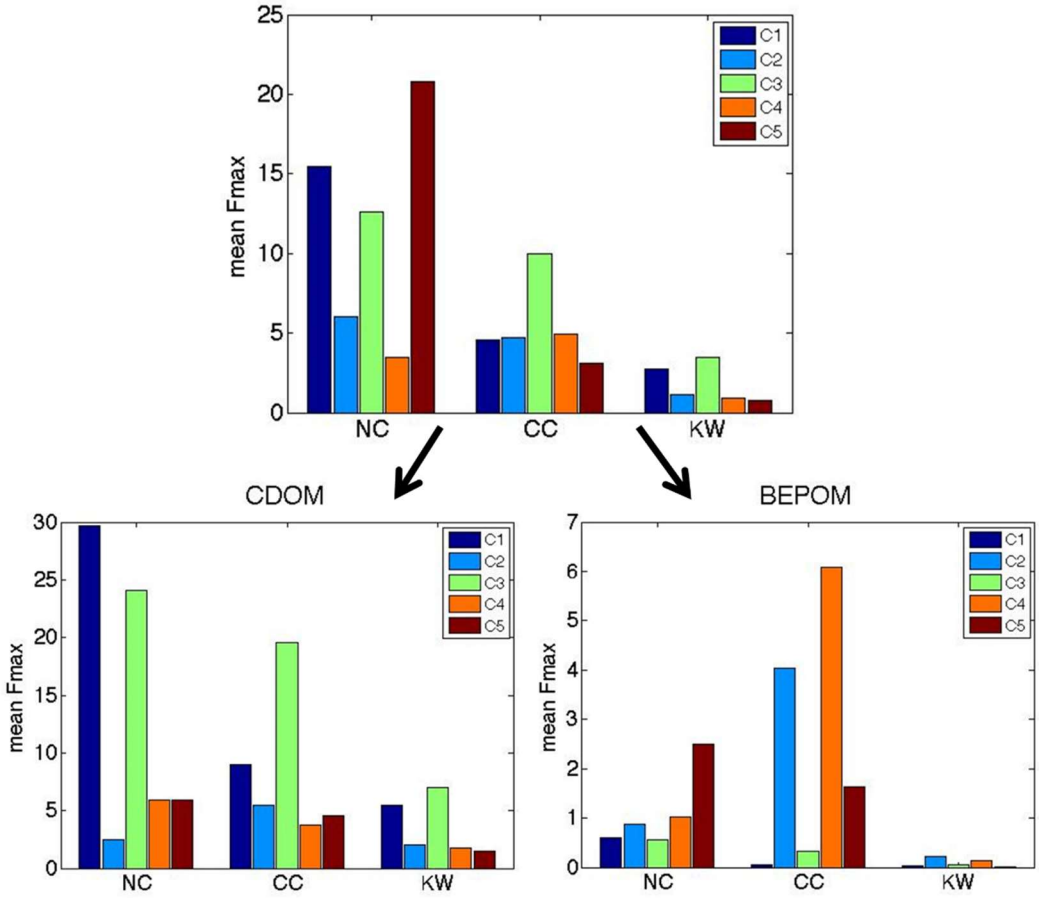


Figure 33. Average fluorescence patterns from the EEM-PARAFAC model used to characterize CDOM (L) and BEPOM (R) from NC, CC, and KW. The top graph averages both carbon pools.

In terms of BEPOM, a very different qualitative pattern emerged. First, North Carolina BEPOM was dominated by C5 and less influenced by C1. The particulates transported in Newport River and adjacent coastal waters therefore appear to be degraded material. In contrast, Corpus Christi was dominated by C2 and C4 fluorescence. Both signals are strongly associated with recent primary production of organic matter (C2) and subsequent degradation by heterotrophic organisms (C4). The latter may not be strictly bacteria and is a matter warranting further study.

Particulates in KW were generally the lowest, and appeared more similar to Corpus Christi suggesting that most of the aromatic OM in that region was dominated by mangroves. Seagrass beds were not studied discretely in this project but could be an ephemeral source of aromatic material. DOM from seagrass beds has been shown in the literature to be quite photolabile and probably is degraded by sunlight in the more transparent coastal waters around Key West.

Aromatic carbon content of coastal waters across three contrasting climates was studied in Y3 using a combination of optical and chemical techniques. Results demonstrated strong riverine forcing of coastal water aromatic C content that dominated over so-called "blue carbon" sources of aromatic DOM such as mangroves. Climate was a key influence on the quality of the organic matter found in Corpus Christi compared to North Carolina or Key West. Good predictability of

the DOC found in Y2 also applied to Key West and Corpus Christi in Y3. Calibration of these signals with geochemical markers of aromatic matter using fluorescence, stable isotopes, and dissolved lignin established the patterns of this tracer. Results were consistent for the Newport River comparing August 2015 to two hydrodynamically distinct periods in August and November 2014, indicating that the technique is applicable for a specific system despite variations in aromatic C concentrations that complicate simply using salinity as a conservative tracer for freshwater. DOM dominated the aromatic organic load in these systems in comparison to POM. For the latter, recent sources of aromatic could include primary production by phytoplankton and subsequent processing by heterotrophs. Aromatic DOM sources in drier environments such as Corpus Christi are effectively concentrated by evaporation increasing the load and the aromaticity of organic matter exported into these coastal waters.

Rate Survey of DoD and Reference Sites Several stations were repeatedly sampled during the four surveys (*e.g.,* KW-1, 2, 5, 6, 15) around coastal NAVFAC sites in Key West and Boca Chica during 2013-2015. Bacterial production was generally similar amongst the different samplings at the same station with the exception of No Name Key (KW-15) at August and November samplings, the former of which was immediately preceded by a substantial rain event. Same station bacterial production was generally less rapid in October 2015 (Table 20). Phenanthrene mineralization was most frequently measured in water samples with detects in 21 of 24 samples though it was only detected in two of three October 2015 sediment samples (Table 20). Most rapid rates of TNT (KW-3), RDX (KW-1) and HMX (KW-1) were all measured in surface sediment. RDX mineralization was most commonly measured in sediment (all four 4 samples; ranged 76-690 $\mu g\ kg^{-1}\ d^{-1}$) and rates were among the most rapid we have reported for natural assemblages (previously 46 $\mu g\ kg^{-1}\ d^{-1}$ for RDX; Montgomery et al. 2012).

To account for vagaries in mineralization rates that are a simple function of bacterial growth rate differences, mineralization rates were normalized to bacterial production and then multiplied by 100 to obtain mineralization as a percent of production. Highest percentages are indicative of bacterial assemblages that are more adapted to preferentially biodegrading that particular carbon substrate. During the May 2013 sampling, TNT mineralization to production percentages were highest in sediment at Boca Chica shallows (KW-3) followed by water at Trumbo Point (KW-6) and overlying water at Boca Chica (Table 21). Percentage of phenanthrene mineralization was highest offshore (KW-2). Both RDX and HMX mineralization to production ratios were highest for Sigsbee Park sediment (KW-5). It should be noted that these comparisons are probably most valid when comparing same station rates at different times or amongst different mixing treatments at the same time.

During the August 2013 sampling, highest ratio of mineralization to bacterial production for both phenanthrene and HMX was found in open ocean water at the offshore station (KW-2) and the *Sargassum* front (KW-F1, -F2, -F3; Table 22). Highest ratios for RDX were amongst sediment samples while the highest HMX ratio was also in sediment (off Fleming Key, KW-1). Ratio of TNT mineralization to production showed little pattern across sites but ranged from 0.02 in the mangrove lagoon to 602 offshore. During the November 2013 sampling, phenanthrene appeared to be mineralized preferentially by mangrove lagoon assemblages and especially when there were large dilutions of mangrove water with adjacent open ocean water. HMX was preferentially mineralized in less diluted (*e.g.,* 40, 50%) mangrove water (Table 23). For HMX, it's possible that the mangrove effluent assemblage was skewed towards aromatic degradation

but that DO was too low for efficient mineralization in the unmixed mangrove lagoon. During October 2015 sampling around Key West, phenanthrene mineralization was a lower percentage of bacterial production than during the three previous samplings (range BD-2.17%, Table 24). Such low percentage can result from flux and utilization of competing alternate carbon sources.

Table 20. Survey of bacterial production and mineralization rates of TNT, phenanthrene (P), RDX and HMX for water (µg C L^{-1} d^{-1}) and sediment (µg C kg^{-1} d^{-1}) around Key West, FL, USA during 2013 and 2015. ND = Not Determined, BD = Below Detect.

			Rate (AVG (SD), µg C kg^{-1} d^{-1} or µg C L^{-1} d^{-1})				
			Mineralization				
Station	**Sampling**	**Bacterial Production**	**TNT**	**P**	**RDX**	**HMX**	**Notes**
	MAY '13	67 (6.7)	BD	0.85 (0.36)	BD	0.14 (0.02)	
KW-1	AUG '13	58 (4.9)	9.5 (8.4)	1.82 (0.45)	BD	BD	Fleming Key mangrove
	OCT '15	15 (1.4)	ND	0.041 (0.005)	ND	ND	
	MAY '13	13 (2.0)	BD	1.0 (0.06)	BD	BD	
KW-2	AUG '13	14 (1.2)	0.31 (0.13)	1.91 (0.26)	0.82 (0.57)	BD	Offshore
	OCT '15	7.1 (0.55)	ND	0.074 (0.008)	ND	ND	
KW-F1	AUG '13	13 (3.7)	7.8 (7.3)	2.25 (0.37)	0.78 (0.28)	BD	
KW-F2	AUG '13	14 (1.0)	0.38 (0.35)	2.06 (0.59)	BD	0.43 (0.13)	*Sargassum* front offshore
KW-F3	AUG '13	13 (0.16)	0.59 (0.40)	1.91 (0.57)	BD	BD	
KW-3	MAY '13	26 (1.9)	0.59 (1.04)	BD	0.77 (0.23)	BD	Boca Chica shallows
KW-4	MAY '13	46 (1.6)	BD	1.22 (0.32)	0.76 (0.54)	BD	Channel Key mangrove
	MAY '13	47 (2.8)	BD	BD	BD	0.62 (0.19)	
KW-5	AUG '13	44 (3.4)	BD	1.90 (0.54)	BD	0.51 (0.23)	Sigsbee Park
	OCT '15	26 (0.33)	ND	0.066 (0.020)	ND	ND	
	MAY '13	33 (1.5)	0.98 (0.75)	BD	0.25 (0.16)	0.82 (0.38)	
KW-6	AUG '13	34 (1.7)	BD	2.07 (0.13)	BD	BD	Trumbo Point
	OCT '15	11 (1.2)	ND	0.053 (0.012)	ND	ND	
KW-13	AUG '13	37 (3.5)	BD	1.85 (0.39)	0.25 (0.23)	0.51 (0.37)	
KW-13	OCT '15	14 (1.5)	ND	0.049 (X)	ND	ND	channel W of Fleming
KW-14	AUG '13	15 (2.6)	1.47 (1.16)	1.94 (0.49)	BD	1.17 (0.32)	Key
KW-45	OCT '15	6.4 (0.23)	ND	0.049 (0.032)	ND	ND	
	AUG '13	186 (5.4)	BD	1.21 (0.13)	2.04 (0.31)	2.57 (0.41)	
KW-15	NOV '13	46 (2.1)	BD	2.22 (0.37)	1.84 (0.25)	BD	No Name Key mangrove
	OCT '15	20 (2.7)	ND	0.05 (0.02)	ND	ND	
KW-16	NOV '13	3.5 (0.27)	BD	0.22 (0.13)	0.62 (0.11)	BD	Open water off Long Key
KW-1	AUG '13	335 (85)	2.12 (0.99)	BD	690 (100)	50 (18)	Fleming Key mangrove
KW-1	OCT '15	216 (45)	ND	BD	ND	ND	
KW-3	MAY '13	279 (83)	8.7 (6.5)	BD	76 (58)	BD	Boca Chica shallows
	MAY '13	273 (37)	BD	BD	134 (68)	BD	
KW-5	AUG '13	93 (48)	BD	BD	290 (102)	BD	Sigsbee Park
	OCT '15	23 (0.89)	ND	0.500 (0.364)	ND	ND	
KW-6	OCT'15	102 (16)	ND	0.643 (0.153)	ND	ND	Trumbo Point

Table 21. Mineralization to production (%) for water and sediment samples collected around Key West, FL, USA during May 2013.

		Mineralization/Production (%)				
Station	**Sample**	**TNT**	**P**	**RDX**	**HMX**	**Notes**
KW-1	mixing experiment		1.3		0.21	Fleming Key mangrove
Mix KW-1/2			3.5		1.5	Mixing experiment
KW-2			7.8			Offshore end member
KW-3	surface water	2.3	0.5	3.0		Boca Chica shallows
KW-4		0.05	2.7	1.7	0.33	Channel Key mangrove
KW-5			1.4		1.3	Sigsbee Park
KW-6		3.0	1.9	0.75	2.5	Trumbo Point
KW-3	sediment	3.1		27.2		Boca Chica shallows
KW-5				49.1	2.6	Sigsbee Park

Table 22. Mineralization to production (%) for water and sediment samples collected around Key West, FL, USA during August 2013.

		Bacterial	Mineralization/Production (%)				
Station		**Production**	**TNT**	**P**	**RDX**	**HMX**	**Notes**
KW-1	surface water	58 (4.9)	16.3	3.1	2.0	2.9	Fleming Key mangrove
KW-5		44 (3.4)	0.10	4.3	0	1.2	Sigsbee Park
KW-6		34 (1.7)	0.16	6.1	0.63	1.5	Trumbo Point
KW-13		37 (3.5)	8.8	5.0	0.68	1.4	channel W of Fleming Key
KW-14		15 (2.6)	9.8	12.9	3.1	7.8	channel W of Fleming Key
KW-1	sediment	335 (85)	0.63	0	206	14.8	Fleming Key mangrove
KW-5		93 (48)	0	0.87	312	0	Sigsbee Park
KW-F1	front	13 (3.7)	60.2	17.3	6.0	11.6	Front -- West/ocean side
KW-F2		14 (1.0)	2.7	14.7	0	3.0	Front -- interface
sargassum		134 (3.6)					Front -- sargassum
KW-F3		13 (0.16)	4.5	14.7	17.8	0.34	Front -- East/bay side
KW-15	mixing experiment	186 (5.4)	0.002	0.65	1.1	1.4	No Name Key mangrove
Mix KW-15/2		158 (2.5)	0	1.0	0.015	1.2	Mixing experiment
KW-2		14 (1.2)	2.2	13.7	5.9	3.5	Offshore end member

Table 23. Mineralization to production (%) for water samples collected around Key West, FL, USA during November 2013.

		Bacterial	Mineralization/Production (%)				
Station		**Production**	**TNT**	**P**	**RDX**	**HMX**	**Notes**
KW-15	mixing experiment	46 (2.2)	0.07	4.8	4.0	2.0	No Name Key mangrove
KW-A		40 (1.3)	0.43	4.5	0.7	10.4	50% KW-15
KW-B		34 (1.2)	0.12	6.9	1.8	6.8	40% KW-15
KW-C		30 (3.5)	0.65	6.2	5.0	1.0	30% KW-15
KW-D		24 (0.85)	0.034	10.3	0	2.5	20% KW-15
KW-E		19 (0.44)	0.94	11.2	0	2.9	10% KW-15
KW-16		14 (0.98)	1.01	1.6	4.6	0	Open water (off Long Key)

Table 24. Mineralization to production (%) for water samples collected around Key West, FL, USA during October 2015. BD = Below Detect.

Station	Bacterial Production (μg C L^{-1} d^{-1})	Phenanthrene Mineralization (% Production)	Notes
KW-1	15 (1.4)	0.27	Fleming Key mangrove
KW-2	7.1 (0.55)	1.04	Offshore
KW-5	26 (0.33)	0.25	Sigsbee Park
KW-6	11 (1.2)	0.48	Trumbo Point
KW-13	14 (1.5)	0.35	channel W of Fleming Key
KW-45	6.4 (0.23)	0.77	
KW-15	20 (2.7)	0.25	No Name Key mangrove
KW-1	216 (45)	BD	Fleming Key mangrove
KW-5	23 (0.89)	2.17	Sigsbee Park
KW-6	102 (16)	0.63	Trumbo Point

These rates of contaminant degradation can be a compelling line of evidence against a typical site conceptual model which assumes that surface soil or sediment contaminants can run off with rainfall and negatively impact adjacent waterway sediment. Several NAVFAC Key West sites within the survey area have maximum reported PAH concentrations of 20-26 ppm in soil and sediment. If a rain event were to transport similar PAH concentrations to surface water and sediment in the adjacent waterway, degradation rates measured during these surveys suggest that this amount would be metabolized in ca. 1-21 days. Likewise, nitrogenous energetic (nitroglycerin, NG) concentrations of 0.11-0.25 ppm (that was reported for a Key West range) would be mineralized in <16 hours by surface water and sedimentary assemblages at these NEC mineralization rates. This analysis is consistent with recent published half lives for NG amongst natural soil organic and associated bacterial assemblages (*i.e.,* ca. 3 h to weeks; Bordeleau et al. 2014). Although these time estimates can be affected by other environmental factors (*e.g.,* weather), the magnitude of these turnover times (*i.e.,* hours to weeks) is a line of evidence that contradicts the site conceptual model that these contaminants have existed in the surface soil and sediment for the past 60 years. A more likely model would involve the ambient PAH concentrations resulting from a balance of current day flux to the site (*e.g.,* atmospheric deposition) minus degradation by microbiota and abiotic processes (*e.g.,* photodegradation). Though NG mineralization rates were not specifically measured in this survey, NG biodegradation rates are reported to be more rapid than those of TNT used in this analysis. Based on our previous experience and given the transient nature of NG in the environment, it is more likely that these are NG values are actually false detects due to lower cost chemical analyses (*i.e.,* shouldering with glycerin using FID detection rather than using GC/MS).

Two DoD sites that were located in the study area for Lower Outer Banks, NC were Camp Lejeune along the New River Estuary and a former target range Cat Island (aka Wood Island) in Bogue Sound, NC. The New River Estuary survey taken in April 2014 was performed along with a SERDP DICERP program survey (though note our station numbers are reversed). Bacterial production was highest mid estuary (NC-05A) and lowest at the freshwater and marine end members (NC-01A and NC-08A, respectively) (Table 25). TNT, RDX and HMX

mineralization was highest near the headwaters and confluence of Northeast and Southwest Creeks at Jacksonville, NC. Phenanthrene mineralization was highest near the mouth of New River (Table 25) where one might expect the highest flux of petroleum from surface runoff at Jacksonville.

Table 25. Survey of bacterial production and mineralization rates of TNT, phenanthrene (P), RDX and HMX for water (AVG (SD) μg C L^{-1} d^{-1}) for DICERP transect of New River Estuary, NC, near Camp Lejeune (April 2014).

		Rate (AVG(SD) μg C L^{-1} d^{-1}) Mineralization				
Station	Bacterial Production	TNT	P	RDX	HMX	Notes (salinity), [New River segment]
NC-01A	45 (2.4)	0.53 (0.42)	0.58 (0.15)	1.3 (1.0)	7.0 (3.9)	DCERP-8 NE Creek (6.9), [M52]
NC-02A	78 (20)	3.4 (0.88)	0.41 (0.21)	4.6 (0.69)	5.1 (1.8)	DCERP-7 JAX (6.4), [JAX]
NC-03A	68 (4.4)	1.1 (0.68)	0.44 (0.07)	1.7 (0.42)	1.8 (0.17)	DCERP-6 SW Creek (6.4), [M52]
NC-04A	89 (22)	0	0.31 (0.28)	4.0 (0.38)	2.6 (1.5)	DCERP-5 Morgan/Farnell Bays (8.0), [M47]
NC-05A	98 (8.5)	0	0.24 (0.24)	3.8 (1.5)	0	DCERP-4 Lejeune (13), [M39]
NC-06A	54 (2.2)	0.40 (0.09)	0.40 (0.22)	2.7 (2.4)	0	DCERP-3 Stones Bay (16.3), [M32]
NC-07A	43 (2.3)	0.35 (0.11)	1.2 (0.05)	2.3 (1.2)	0	DCERP-2 Courthouse Bay (27.2), [M18]
NC-08A	16 (1.1)	0	0.35 (0.04)	0.64 (0.33)	0	DCERP-1 New River Inlet (34.9) , [M15]

The second DoD relevant site within the Lower Outer Banks study area was Bogue Sound, NC, that included the former target range site of Cat Island in the southwestern portion of the Sound off of Emerald Isle. Bogue Sound samples were taken across the sound from Cat Island (NC-01), near an oyster bar on the western edge of the Sound at Emerald Isle (NC-02) and on the eastern edge of the Sound across from Morehead City near Beaufort Inlet and the UNC lab (NC-03; Figure 5). Surface water was collected during all three samplings during 2014 but sediment was only collected during the April and November. Bacterial production ranged from 7.8-74 μg C L^{-1} d^{-1} in the surface water and 58-551 μg C L^{-1} d^{-1} in the sediment and was generally lowest in November. Surface water mineralization rates ranged from BD-0.21 μg C L^{-1} d^{-1} for TNT, 0.10-0.83 μg C L^{-1} d^{-1} for phenanthrene, BD-7.5 μg C L^{-1} d^{-1} for RDX, and BD-14.5 μg C L^{-1} d^{-1} for HMX (Table 26). Sediment mineralization rates ranged from 1.78-25 μg C kg^{-1} d^{-1} for TNT, BD-1.3 μg C kg^{-1} d^{-1} for phenanthrene, 306-1099 μg C kg^{-1} d^{-1} for RDX, and 32-105 μg C kg^{-1} d^{-1} for HMX (Table 26). These Cat Island sediment mineralization rates for RDX were among the highest found for any natural ecosystem media (even higher than found at Key West in 2013) and they rivaled those found in mesocosm systems with continuous input of RDX (Table 28).

The Newport River Estuary salinity transect was surveyed again in August 2015 from the Cypress bog (PSU = 0.07) in Newport, NC to the Radio Island boat launch by Beaufort Inlet (PSU = 35.90). Mineralization of both TNT and phenanthrene were highest in the shallow lower estuary (PSU = 21-31) where there is a lot of wind-driven mixing (Figure 34). This is also where dissolved oxygen increases dramatically from 6.83 to 10.9 mg L^{-1} which could be the result of mixing of atmospheric O_2 into the estuarine water or from a phytoplankton bloom (Figure 35). However a phytoplankton bloom would likely stimulate heterotrophic bacterial production which actually decreases in this zone (Figure 36). There is some relationship between DO and TNT mineralization but it is weak (r^2 = 0.46; Figure 37). Interestingly, the highest rate of phenanthrene mineralization during this survey in the well mixed lower estuary (NC-121; 0.53 μg L^{-1} d^{-1}) was similar to the highest rate in the mixing experiment (0.60 μg L^{-1} d^{-1}) and at the Bear Island turbidity front interface (0.82 μg L^{-1} d^{-1}).

Table 26. Bacterial production and mineralization rates (AVG (SD) µg C L^{-1} d^{-1} or µg C kg^{-1} d^{-1}) for water and sediment from Bogue Sound, NC (CY14-15).

		Rate (AVG(SD) µg C L^{-1} d^{-1} or µg C kg^{-1} d^{-1})									
		Bacterial Production	Mineralization				Mineralization/Production (%)				
Station	Sampling		TNT	P	RDX	HMX	TNT	P	RDX	HMX	Notes (salinity)
NC01	APR '14	61 (3.1)	0.21 (0.10)	0.17 (0.17)	1.9 (1.2)	4.9 (2.3)	0.34	0.27	3.1	8.0	Cat Is. water (32)
	AUG '14	74 (4.1)	BD	0.33 (0.14)	6.2 (1.5)	11 (3.0)	0	0.44	8.3	15	(17.1)
	NOV '14	7.8 (1.2)	BD	0.17 (0.05)	BD	BD	0	2.2	0	0	
	AUG '15	24 (1.4)	ND	ND	ND	ND	ND	ND	ND	ND	(32.8)
NC02	APR '14	62 (7.8)	BD	0.41 (0.02)	1.2 (0.30)	BD	0	0.66	1.9	0	Emerald Is. water (ND)
	AUG '14	35 (1.7)	BD	0.83 (0.32)	3.7 (2.4)	8.2 (2.1)	0	2.4	11	24	(24)
	NOV '14	8.8 (0.91)	0.18 (0.07)	0.10 (ND)	6.7 (2.0)	14.5 (2.8)	2.0	1.1	76	165	
	AUG '15	39 (1.5)	ND	ND	ND	ND	ND	ND	ND	ND	(33.4)
NC03	APR '14	42 (1.1)	BD	0.34 (0.14)	0.79 (0.32)	1.1 (0.50)	0	0.80	1.9	2.6	Beaufort Inlet water (ND)
	AUG '14	31 (0.60)	0.11 (0.10)	1.3 (0.07)	BD	3.5 (1.3)	0.35	4.2	0	11	(27.5)
	NOV '14	8.2 (0.74)	BD	0.013 (0.006)	7.5 (1.6)	3.7 (2.7)	0	0.15	91	45	
NC01	APR '14	551 (101)	15 (1.8)	BD	884 (305)	68 (23)	2.7	0	160	12	Cat Is. sediment
	NOV '14	58 (28)	5.1 (0.55)	0.18 (0.15)	1099 (590)	104 (11)	8.8	0.31	1889	178	
	AUG '15	126 (10)	2.91 (0.06)	BD	ND	ND	2.3	0	ND	ND	
NC02	APR '14	281 (126)	25 (16)	BD	699 (278)	75 (16)	8.9	0	249	27	Emerald Is. sediment
	NOV '14	157 (13)	5.8 (0.50)	0.30 (0.037)	785 (288)	105 (24)	3.7	0.19	500	67	
	AUG '15	211 (33)	1.78 (1.95)	0.61 (0.48)	ND	ND	0.84	0.29	ND	ND	
NC03	APR '14	362 (28)	9.2 (0.05)	0.38 (0.02)	306 (59)	32 (9.6)	2.5	0.10	85	8.8	Beaufort Inlet sediment
	NOV '14	213 (16)	6.3 (2.1)	1.3 (0.79)	371 (193)	56 (11)	3.0	0.59	175	27	

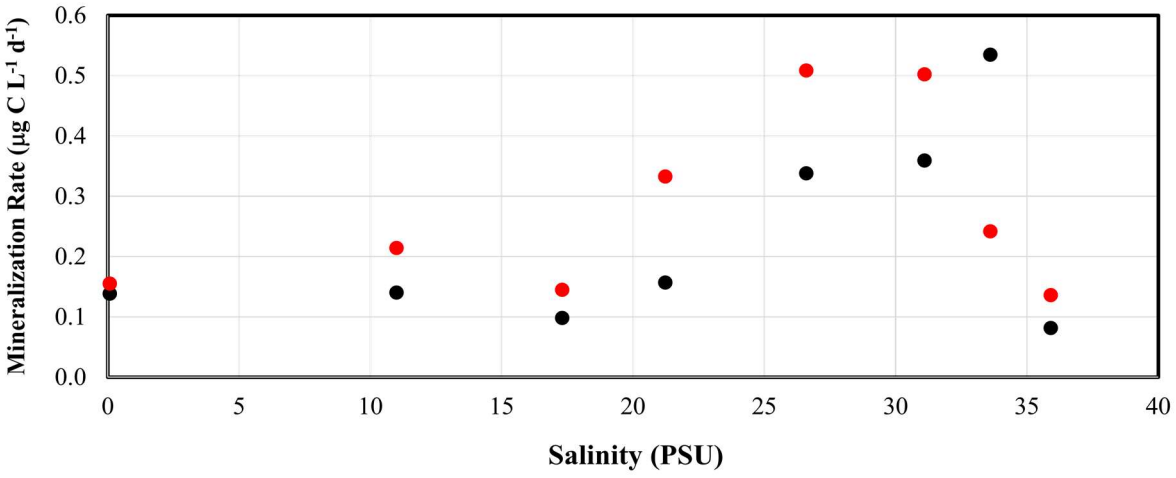


Figure 34. Mineralization rate (µg C L^{-1} d^{-1}) of TNT (red) and phenanthrene (black) along a salinity transect of Newport River Estuary in August 2015.

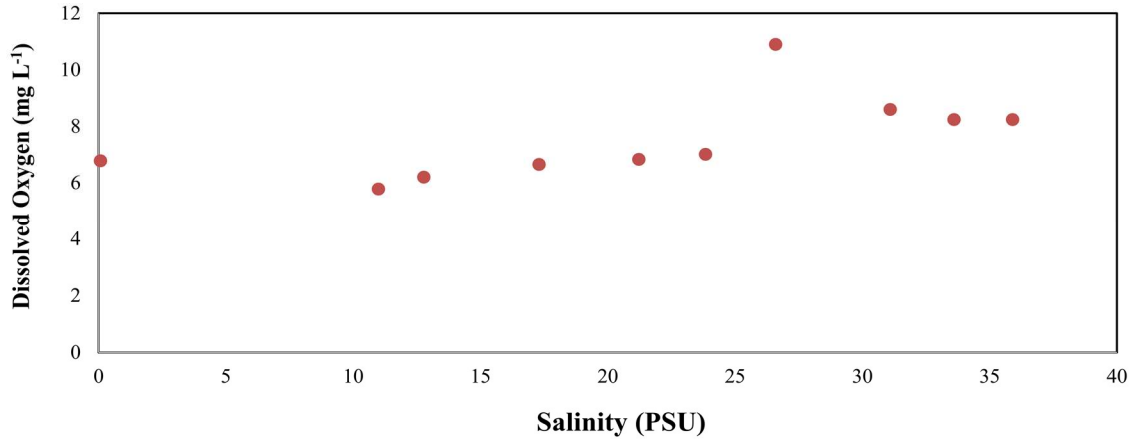


Figure 35. Dissolved Oxygen (mg L^{-1}) along a salinity transect of Newport River Estuary in August 2015.

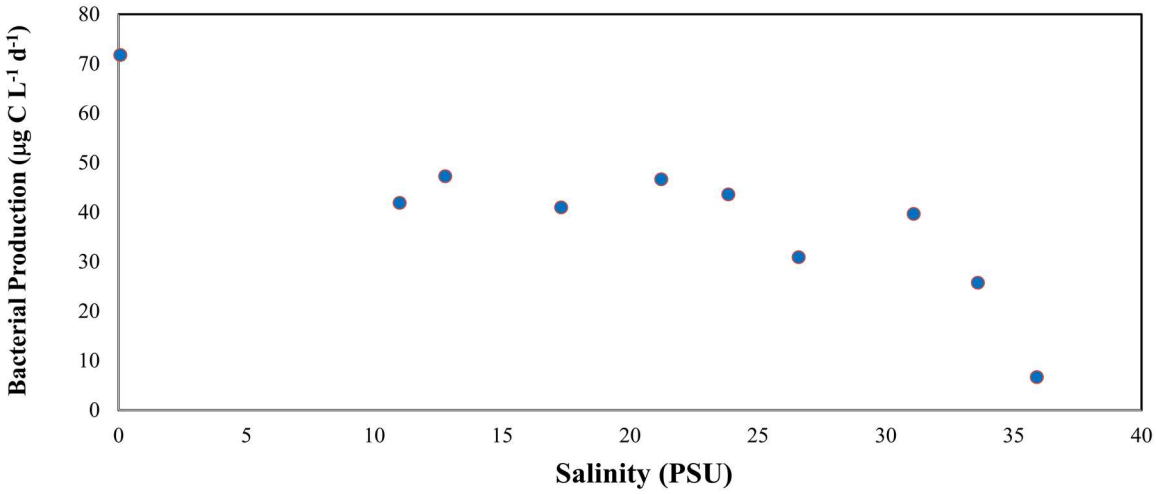


Figure 36. Bacteria production rate (µg C L^{-1} d^{-1}) along a salinity transect of Newport River Estuary in August 2015.

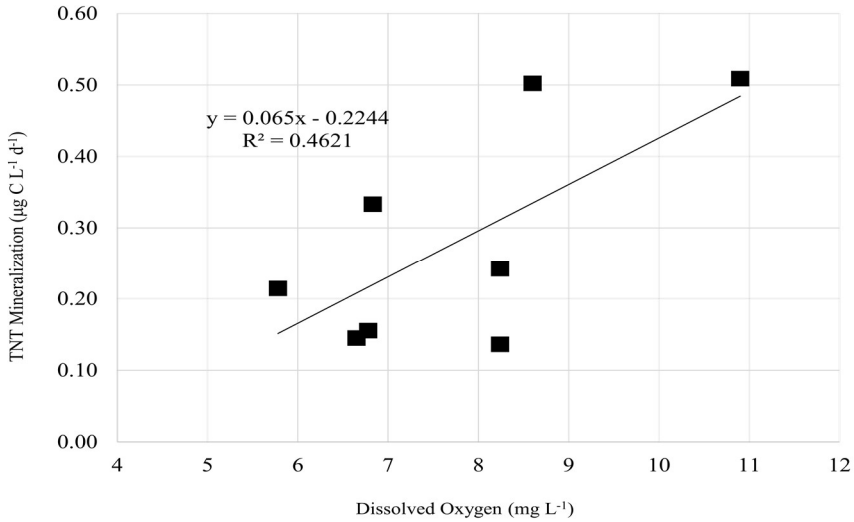


Figure 37. TNT mineralization rate (µg C L^{-1} d^{-1}) verses Dissolved Oxygen (mg L^{-1}) for Newport River Estuary in August 2015.

In September 2015, Corpus Christi Estuarine System was sampled as this ecosystem represents a subtropical wet biome type where we have no historical data. Any information collected here could be useful in evaluating MRP sites at Corpus Christi Naval Air Station which is adjacent to both Corpus Christi and Oso Bays. The sampling was abbreviated because of small craft advisories, as well as, a red tide bloom. Bacterial production and TNT mineralization rates were most rapid at the Corpus Christi Bay station (CC-3; 387+/-32 and 0.204+/-0.100 μg C L^{-1} d^{-1}, respectively) (Table 27). Phenanthrene mineralization was most rapid (0.077+/-0.028 μg C L^{-1} d^{-1}) in a channel draining a mangrove flat (CC-5) where bacterial production was second highest (Table 27). Bacterial production and mineralization of both TNT and phenanthrene were least rapid in the eutrophic Oso River feeding Oso Bay (CC-1; 168+/-20, 0.038+/-0.013, and 0.033+/-0.006 μg C L^{-1} d^{-1}, respectively). TNT and phenanthrene mineralization rates were in a similar range to that of the Lower Outer Banks, NC and coastal Key West, FL, even though bacterial production was 2 to 10-fold higher in the Corpus Christi System. It is possible that autochthonous carbon produced as a result of the red tide and high nutrient input from recent rains stimulated bacterial production with competing organic carbon (Vernet and Whitehead 1996).

Table 27. Rates of bacterial production and mineralization of TNT and phenanthrene (P; μg C L^{-1} d^{-1}) during the September 2015 survey of Corpus Christi Bay Estuarine System.

	Rate (AVG(SD) μg C L^{-1} d^{-1})					
		Mineralization				
Station	Bacterial Production	TNT	TNT (% of production)	P	P (% of production)	Notes (salinity)
CC-1	168 (20)	0.038 (0.013)	0.02	0.033 (0.006)	0.02	Oso River (2.76)
CC-2	170 (8)	0.104 (0.104)	0.06	0.047 (0.005)	0.03	Aransas Pass (31.9)
CC-3	387 (32)	0.204 (0.100)	0.05	0.060 (0.017)	0.02	Corpus Christi Bay (32.0)
CC-4	250 (32)	0.079 (0.003)	0.03	0.040 (0.007)	0.02	Mangrove Flat (41.9)
CC-5	191 (23)	0.082 (0.024)	0.04	0.077 (0.028)	0.04	Channel (33.5)

ER-2122 Mesocosm (POC: C. Tobias) In collaboration with SERDP project ER-2122 (Craig Tobias) "Tracking the Uptake, Translocation, Cycling, and Metabolism of Munitions Compounds in Coastal Marine Ecosystems using Stable Isotopic Tracer", samples from media taken from mesocosms housed at the University of Connecticut were processed for bacterial production and mineralization of TNT, RDX, and phenanthrene. These data from three mesocosm experiments (August and October 2013 and June/July 2014; Ballentine et al. 2016, Smith et al. 2013, 2015) were provided to Drs. Tobias and Penny Vlahos to ground truth their mass loss estimates of TNT and RDX within these simulated intertidal ecosystems (Table 28). Full analyses of this data will be a part of the ER-2122 reporting, but mineralization rates for TNT and RDX in surface sediment were much higher than those found in natural systems (TNT: 1066 +/- 165 μg C kg^{-1} d^{-1}; RDX: 7411 +/- 2092 μg C kg^{-1} d^{-1}, Table 28). These mesocosm experiments demonstrate that natural estuarine assemblages can adapt to continuous energetic input and dramatically increase TNT and RDI biodegradation in sediment. By extension, our maximum ecosystem capacity estimates based on ambient mineralization rates (*e.g.,* water and sediment unexposed to elevated contaminant levels) would likely under estimate the capacity of an ecosystem receiving chronic, elevated energetic input.

Table 28. Bacterial production and mineralization rates (AVG (SD) µg C L^{-1} d^{-1} or µg C kg^{-1} d^{-1}) for mesocosm samples performed in collaboration with ER-2122 (POC: Craig Tobias) in August, October 2013 and June, July 2014. ND = Not Determined, BD = Below Detect.

			Rate (AVG (SD) µg C kg^{-1} d^{-1}, µg C L^{-1} d^{-1})			
Sampling	**Mesocosm**	**Media**	**Bacterial Production**	**Mineralization TNT**	**Phenanthrene**	**RDX**
T_f (AUG13)	TNT	water	26 (6.9)	BD	BD	ND
		foam	25 (2.4)	BD	BD	ND
		sediment (oxic)	233 (43)	BD	0.60 (0.23)	ND
		sediment (anoxic)	266 (64)	BD	3.53 (0.64)	ND
	RDX	water	74 (4.2)	ND	BD	5.94 (1.51)
		foam	20 (4.3)	ND	BD	1569 (255)
		sediment (oxic)	251 (20)	ND	1.03 (0.51)	1864 (448)
		sediment (anoxic)	247 (31)	ND	2.22 (0.90)	1066 (252)
T_f (OCT 13)	TNT	water	12 (3)	1.7 (3.1)	ND	ND
		foam	811 (--)	1.0 (0.9)	ND	ND
		sediment (oxic)	146 (62)	8.5 (2.9)	ND	ND
		sediment (anoxic)	173 (12)	1066 (165)	ND	ND
	RDX	water	60 (9)	ND	ND	6.0 (1.3)
		foam	170 (199)	ND	ND	487 (242)
		sediment (oxic)	129 (23)	ND	ND	48 (8)
		sediment (anoxic)	160 (34)	ND	ND	2317 (285)
T_0 (17JUN14)	Baseline	sediment (oxic)	1885 (373)	13.2 (1.47)	16.1 (0.22)	1010 (190)
T_f (10JUL14)	TNT	sediment (oxic)	838 (333)	6.66 (1.32)	3.76 (2.22)	7411 (2092)
	RDX	sediment (oxic)	960 (136)	28.7 (13.0)	0.41 (0.24)	5398(1818)
T_{f+1} (18JUL14)	TNT	foam	8 (19)	4.20 (0.23)	ND	96 (11)
		sediment (oxic)	841 (57)	27.0 (14.4)	ND	2619 (522)
	RDX	foam	165 (14)	ND	ND	98 (4)
		sediment (oxic)	693 (57)	5.96 (0.01)	ND	3201 (902)

Effect of Bioremediation Amendments The effect of two bioremediation amendments, Provectus-CH4 and Provectus-IR, on heterotrophic bacterial production, as well as, mineralization of TNT and RDX was also examined. The Archaeal cell wall inhibitor, lovastatin (in Provectus-CH4), inhibited heterotrophic bacterial production at high concentration (125 ppm) but had little effect or may have even been stimulatory at lower concentration (<50 ppm; Figure 38) using water collected from a Delaware marsh creek (DE-2, September 2014; Figure 7). At 50 ppm, the statin alone had little effect on RDX mineralization using that same marsh creek water (Table 29); however, the bioremediation amendment, Provect-IR (50 ppm) appeared to increase RDX mineralization rate in the surface water sample by about two-fold (Table 29). Note the Provect-IR contains the statin, as well. The effect of Provect-IR (6.25% addition by sediment dry weight) on TNT and RDX mineralization in estuarine surface sediment was also measured (Bogue Sound, NC, November 2014; Figure 5). Although there was no effect on TNT mineralization (data not shown), RDX mineralization was enhanced ca. 4-14 fold above ambient rates of biodegradation (Table 29**)**. RDX mineralization rates with Provect-IR (6.25%) were very similar to those found upon continuous expose to RDX in the ER-2122 mesocosm experiment (4369-5208 vs. 1066-5398 µg kg^{-1} d^{-1}, respectively).

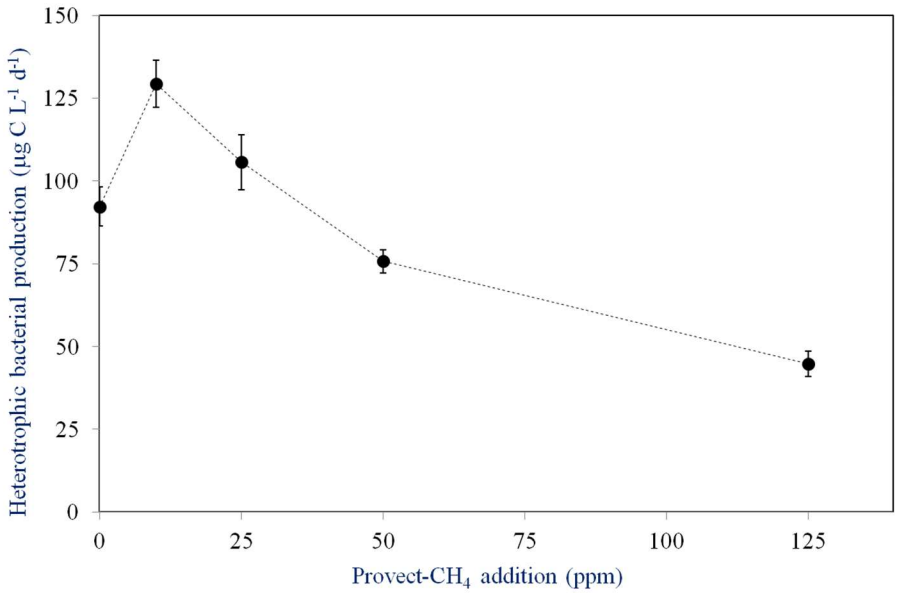


Figure 38. Effect of the statin, Provect-CH4 (ppm), on rate of heterotrophic bacterial production (AVG (SD) µg C L^{-1} d^{-1}).

Table 29. Provectus-CH4 in marsh creek water (Canary Creek, DE; September 2014) and surface sediment (Bogue Sound, NC; November 2014).

Estuarine Media	Sample	Amendment	RDX Mineralization (AVG (SD) µg kg^{-1} d^{-1} or µg L^{-1} d^{-1})
Water	DE-2	None	3.5 (0.36)
		Provect-CH4 (50 ppm)	3.1 (0.46)
		Provect-IR (50 ppm)	7.7 (1.8)
Sediment	NC-1	None	1099 (590)
		Provect-IR (6.25%)	4369 (2890)
	NC-2	None	785 (288)
		Provect-IR (6.25%)	4717 (2400)
	NC-3	None	371 (193)
		Provect-IR (6.25%)	5208 (1586)

Biogeochemistry Table Deliverable Transferring the information from this project into a format that the end user (*e.g.,* NAVFAC site RPM) can use in a remedial site investigation is an important deliverable. The data from this study of tropical (wet), subtropical (wet) and temperate ecosystems has been divided into salinity (*i.e.* fresh/brackish, estuarine, and marine) and dissolved oxygen regimes (low and high DO) and presented as a range (Table 21). Both salinity and DO are surface water quality properties that are routinely measured as part of remedial site investigations and discharge permits. Numerical values represent the range of detectable average mineralization rates found under specific salinity and DO conditions during surveys at coastal Key West (Tropical wet), Corpus Christi Bay (Subtropical wet), and Lower Outer Banks (Temperate). Another way of presenting this type of data would be as average rates for all measured samples within each regime; however, providing data range may give a more complete picture of findings for stakeholders and regulators. Energetics biodegradation rate data for a particular ecosystem can be provided to site RPMs through technical support staff (*e.g.,* EXWC) or can be found in the published peer-reviewed literature. It is possible that this type of

information (with a hyperlink to the peer-reviewed reference) can be made into a phone or tablet application for ease of access by end users. However the ecosystem capacity deliverable may actually preclude the importance of this deliverable (see below).

Ecosystem Capacity Deliverable Based on the range of biodegradation rate data collected in this project for salinity transects of the New River, NC (Table 30), capacity for the Newport River Estuarine system to metabolize a hypothetical release of contaminants was estimated (Table 31). Degradation rate ranges are coupled to volume of a given media within the ecosystem (in this case, water) and expected residence time for transit through the study area. The Newport River Estuary is a well mixed, tidal system with a surface area of 453 km^2, an average depth of 1 m and a typical residence time of six days (12 tidal cycles; Couilliette and Nobel 2008 and references therein). For example, a TNT release of 0.3 to 9.2 kg at the Cypress bog in Newport, NC, could be metabolized in the water column during the expected six-day residence time in the estuary prior to any energetic parent compound reaching Beaufort Inlet. This capacity estimate would be for the water column only as sediment data was not collected for this system. As more published data is compiled under a wider variety of conditions (and perhaps with climate change), ecosystem capacity estimates become more predictive of future conditions.

Table 30. Mineralization rate ranges (μg C L^{-1} d^{-1}) for detectible rates of biodegradation are presented for coastal waters based on salinity (PSU) and Dissolved Oxygen (% saturation). Low DO for freshwater/brackish environments is <50% and for estuarine and marine environments is <90%. ND = Not Determined (because of the low freshwater input to the system; sample type not found).

		Mineralization Rate (range, μg C L^{-1} d^{-1})							
		TNT		Phenanthrene		RDX		HMX	
Ecosystem	Salinity (PSU)	low DO	high DO	low DO	high DO	low DO	high DO	low DO	high DO
Temperate	Fresh/Brackish (<5)	0.19-0.20	0.14	0.036-0.45	0.17-0.4	1.8-10.6	3.1-5.3	8.9-11.7	4.0-14
	Estuarine (5-29.9)	0.15-0.22	0.11-3.4	0.068-0.83	0.1-1.3	0.86-5.0	1.3-6.7	4.3-8.3	1.8-14.5
	Marine (30-35)	0.16-1.61	0.21	0.24-0.98	0.013-0.35	3.7-7.0	0.64-7.5	6.3-8.1	0.1-3.7
Tropical (Wet)	Fresh/Brackish (<5)	ND	ND	ND	ND	ND	ND	ND	ND
	Estuarine (5-29.9)	ND	ND	ND	ND	ND	ND	ND	ND
	Marine (30-35)	0	0.31-1.5	1.2-2.2	0.22-2.23	1.8-2.0	0.25-0.82	2.6	0.14-1.2

Ecosystem capacity was also determined using New River Estuary survey data collected along with the DICERP study in April 2014, though this only included a single data collection so the average for each estuary segment was used rather than a range for the entire water body. Segment designations (Figure 39), water volumes and residence times for each portion of the estuary (Table 32) were taken from Ensign et al. (2004). Both volume (1.3 x 10^{11} L) and mean residence time (70 d) were significantly longer than those for the Newport River so the capacity is much greater. Total amount of compound biodegradation for TNT (446 kg), phenanthrene (656 kg), RDX (5748 kg), and HMX (1513 kg) represents the mass mineralized for a hypothetical release in headwaters at Jacksonville, NC, given the average residence time for each river sector. Amount calculated for a hypothetical release at Camp Lejeune into the adjacent New River (sum of the values for sectors below the dashed line in Table 23) is 298 kg of TNT, 481 kg of phenanthrene, and 4223 kg of RDX. That for HMX is listed as 0 kg for a Camp

Lejeune release because HMX mineralization was below the detection limit of <0.01 μg C L^{-1} d^{-1}, however, it would be more accurate to list this as <14 kg based on this detection limit placed into the capacity calculations. Large changes in freshwater input would be expected to reduce ecosystem capacity through reduced retention times but there could be other effects that are compound specific. Phenanthrene mineralization could be additionally reduced as freshwater assemblages would comprise a larger proportion of the estuary with increased flow and the survey indicated more rapid mineralization with marine or higher salinity estuarine assemblages. Conversely, HMX mineralization was much more rapid at salinities below 8, so higher freshwater input may increase HMX mineralization to lower reaches of the estuary.

Table 31. Ecosystem capacity for biodegradation of contaminants (kg) released at headwaters of Newport River Estuary, NC, USA (water volume, 4.53 x 10^8 L; residence time, 6 days) based on range of mineralization data (μg C L^{-1} d^{-1}) collected for that system in 2014.

	Mineralization Rate (range, μg C L^{-1} d^{-1})		Newport River Estuary Capacity (kg, 6 days)	
Contaminant	**low**	**high**	**low**	**high**
TNT	0.11	3.4	0.30	9.2
Phenanthrene	0.013	1.3	0.035	3.5
RDX	0.64	10.6	1.7	29
HMX	0.1	14.5	0.27	39

Table 32. Ecosystem capacity for biodegradation of contaminants (kg) released at headwaters of New River Estuary, NC, USA (water volume, 1.3 x 10^{11} L; AVG residence time, 70 days) for separate sectors (Ensign et al. 2004) based on mineralization rate data (converted to kg d^{-1}) for volume of water in each sector. Below the dashed line represents capacities for sectors down estuary of Camp Lejeune. Based on the measured bacterial production for this same transect, the total metabolic capacity of carbon was 49,096 kg 70 d^{-1}.

New River Section	Volume (L)	AVG Residence Time (d)	Biodegradation (kg d^{-1})				Biodegradation (kg) at AVG Residence Time			
			TNT	P	RDX	HMX	TNT	P	RDX	HMX
JAX	4.06E+09	2	14	2	19	21	30	4	41	45
M52	1.E+10	9	14	8	25	71	118	73	214	624
M47	2.44E+10	13	0	7	97	64	0	98	1269	844
M39	3.25E+10	18	0	8	124	0	0	139	2169	0
M32 (+172)	3.66E+10	20	14	15	99	0	285	288	1950	0
M18	8.13E+09	4	3	10	18	0	13	42	81	0
M15	8.13E+09	4	0	3	5	0	0	13	23	0
Total	1.3E+11	70	45	53	387	156	446	656	5748	1513

Finally, ecosystem capacity was determined for the shallow, lagoon estuary Bogue Sound in Lower Outer Banks, NC, which included Cat Island. This is the only one of these ecosystem analyses that includes sediment capacity along with water column capacity. Sediment volume calculations use sediment surface area that is 1 cm deep so a mass of 2.7 x 10^9 kg, as well as, a water volume of 3.6 x 10^{11} L and an average resident time within this shallow, wind and tidal mixed estuary of 2 days (NOAA 2015). Though most phenanthrene and HMX mass removal would occur in the water column, TNT and RDX were removed predominantly by surface sediment assemblages (Table 33). Relative to the Newport and New Rivers, there is relatively much less freshwater input into Bogue Sound. Mass of energetics removed per day in Bogue Sound is roughly equivalent to that found in 300 twenty-four inch ordnances (*i.e.,* 8 kg each) but it assumes that the energetic compounds are evenly dispersed throughout estuarine media.

Documenting and including mass biodegraded at frontal boundary areas (related to an actual release for a specific time frame) would also increase ecosystem capacity. The volume of these areas were not a part of this published watershed information so it was not included here.

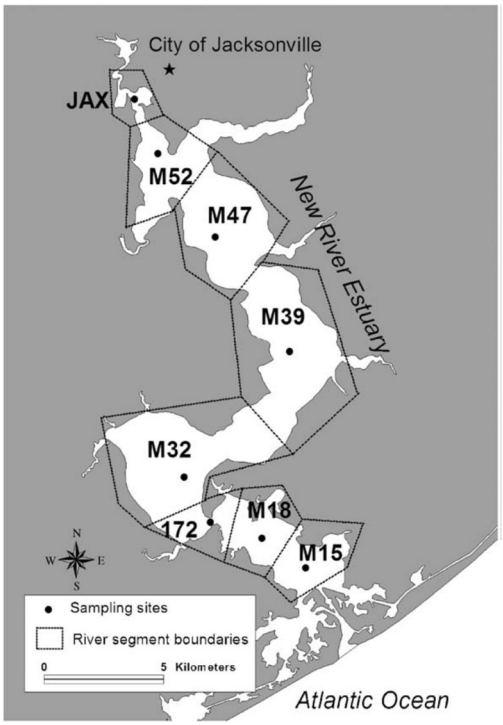


Figure 39. River segments of New River Estuary, NC, USA taken from Ensign et al. (2004) were used to calculate water volumes and residence times.

Table 33. Ecosystem capacity for biodegradation of contaminants (kg) released into Bogue Sound, NC, USA (water volume, 3.6 x 10^{11} L; sediment mass (top 1 cm), 2.7 x 10^{9} kg; AVG residence time, 2 days) based on the AVG mineralization rate for water (μg C L^{-1} d^{-1}) and sediment (μg C kg^{-1} d^{-1}). Bogue Sound sampling included a station by the former target range Cat Island (aka Wood Island) during CY14.

	AVG Mineralization Rate		Bogue Sound Capacity (kg, 2 d)		
Contaminant	**μg C L^{-1} d^{-1}**	**μg C kg^{-1} d^{-1}**	**water**	**sediment**	**Total**
TNT	0.056	11	20	30	50
Phenanthrene	0.41	0.36	147	1.0	147
RDX	3.1	691	1120	1866	2985
HMX	5.2	73	1876	197	2073

This exercise uses the range of detectable rates of mineralization and is likely conservative as it does not take into account the amount of parent compound carbon expected to be incorporated into bacterial biomass (ca. one order of magnitude more than the amount mineralized; Montgomery et al. 2011a) or increased biodegradation occurring at frontal boundaries. ***It demonstrates the potential usefulness of these calculations to a site RPM when trying to determine the potential impacts of energetics or PAH release into an adjacent coastal water body***. This type of information can be particularly useful for mitigating impacts of active range use on the local ecosystem. Impacts would be minimized if surface runoff of energetics (or PAHs or other anthropogenic organic) were limited to below the carrying capacity for a given ecosystem. For compounds where DoD sites are the only source, the calculation is more

straightforward than for more common organics, such as PAHs, that have multiple sources (as determined by a Watershed Source Document). Future ecosystem capacities will also vary due to climate change as local rainfall, wind, and temperature change will all affect estuarine residence time and degradation rates.

We conclude that this ecosystem capacity deliverable is more scientifically defensible and thus more useful to the site RPM than our original deliverable of providing a range of biochemical factors that could be related to a range of energetic biodegradation rates across ecosystems (e.g. Table 1). This is because the ecosystem capacity deliverable provides an actual snapshot of the ecosystem to assimilate a theoretical or known release. Though seasonal and spatial variation in biodegradation rates varies by several orders of magnitude, this capacity snapshot can anchor an order of magnitude estimate of contaminant removal. This sort of value would be most useful for explaining why known recent releases are rapidly below detection limit in water and sediment rather than as some metric for permit of future release amounts. This is because a point source shoreside release could cause temporary, elevated concentration-related toxicity or ecosystem changes that were not investigated here.

IMPLICATIONS FOR FUTURE RESEARCH

We found that aromatic contaminants are much more rapidly mineralized and metabolized by natural bacterial assemblage than has been reported for laboratory cultures or results based on the extrapolation of field ambient concentration data (*e.g.* Harrison and Vane 2010, Stenuit and Agathos 2010, Muter 2014). This supports our previous findings based on our sensitive radiotracer methods (Boyd et al. 2008, Montgomery et al. 2004, 2008, 2010, 2011a,b, 2012, 2013). Our findings contrast with longstanding fundamental principles of most bioremediation strategies which contend that these contaminants are "recalcitrant" due primarily to their chemical structure (Hawari et al. 2000, Qasim et al. 2007). Though this work counters most currently accepted bioremediation research, these findings are consistent with the emerging biogeochemically-based understanding of organic matter metabolism in soil, aquatic and marine ecosystems (*e.g.* Marin-Spiotta et al. 2014, Billings et al. 2015, Han et al. 2016). This more scientifically-robust paradigm concludes that organic matter is not poorly metabolized due to chemical structure but is degraded relative to encounter rate with bacteria (Traving et al. 2015) which is thus linked to contaminant and bacterial concentrations in nature (Arrieta et al. 2015; Middelburg 2015) particularly at the microscale (Stocker 2015). These simultaneous advances in organic matter science challenge long held models of inherent chemical recalcitrance (*e.g.* the humification model; Lehmann and Kleber 2015) and have dramatic implications for site investigations, engineering based remedial strategies and DoD site closure. Because it has been assumed that aromatic contaminants, such as TNT and PAHs, are recalcitrant based on their chemical structure, site investigation data and remedial strategies have been evaluated through this paradigm. It is imperative that future research be conducted based on this emerging science rather than to continue to accrue remedy failures based on prior understandings of recalcitrance and environmental organic carbon metabolism (Nadeau and Scaggs 2007, ASTSWMO 2013). We have four suggestions to improve the outcomes of future SERDP bioremediation research:

1) Revisit the results of previous SERDP sponsored bioremediation strategies that were based on the inappropriate paradigms. For instance, if PAHs are believed to be persistent in

surface sediment because of their chemical structure (as is suggested based on similar surface sediment concentrations over time in field samples), but ambient levels are actually too dilute to support adequate microbial encounter rates (ppm; Arrietta et al. 2014), then current sources rather than historical input may be the controlling factor in PAH persistence. This realization, based on state-of-the-art knowledge in environmental chemistry and microbiology, would call into question the efficacy of any strategy designed to remediate historically contaminated surface sediment (*e.g.* activated charcoal, capping) and likely result in "recontamination" (see examples in Table 1, ASTSWMO 2013).

2) Update standard methodology for field study to include actual measures of bacterial metabolism (^{3}H-leucine method) rather than indirect (but inexpensive) measures, such as change in electron acceptor concentration over time. Despite the obvious importance of understanding relative rate of bacterial carbon metabolism amongst different media, this is rarely measured in bioremediation studies outside of our work.

3) Although end user and environmental contractor input ("service reviews") can be useful to maintain feasibility of implementing solutions, this limits the direction of basic 6.1 research towards that which supports long-standing, often inappropriate paradigms (inherent chemical recalcitrance, etc). There is a tremendous resistance to accepting any new methods or strategies that provide much more accurate information (*e.g.* short-term ("instantaneous") radiotracer techniques verses changes in ambient concentration of organic compounds over time) for fear that these methods will be more expensive or difficult for an environmental contractor or other non-research scientist to interpret. Use of service reviews in the SERDP IPR process should be dramatically reduced.

4) Either solicit greater top level DoD support for performing research at DoD sites or uncouple field implementation of scientific projects from regulatory-guided and ongoing site investigations. Though coupling SERDP-ESTCP projects with ongoing DoD site SIs can be a significant cost saving measure that provides "real world" context for testing potential remedial strategies and a trove of site support data, our experience over the past twenty years is that this adds political and financial context that may be difficult to divorce from the study. This coupling of project to SI tends to limit the use of proper controls (*e.g.* not adding 10K L of unamended groundwater as a control along with 10K L of treatment groundwater to a site to measure the effect of that alone on contaminant concentrations) and due to site legal and regulatory sensitivity, can limit the scientific dissemination of negative or unsuccessful efficacy findings.

CONCLUSIONS

During this three year project, we found that mixing experiments between tropical mangrove lagoon water or Cypress bog water and open ocean water resulted in more rapid rates of bacterial growth aromatic and contaminant mineralization than would have been predicted by interpolation using unmixed samples. This line of evidence supports the hypothesis that coastal mixing zones may stimulate more rapid energetic and PAH degradation than would be expected using standard measures and techniques. Surveys of energetic and PAH mineralization rates in

areas adjacent to DoD sites in Key West suggest that contaminants in surface runoff from shoreside area would be rapidly metabolized (hours to weeks) in the adjacent seawater and surface sediment potentially mitigating ecological risk. Ecosystem capacities for contaminant biodegradation were also determined for three North Carolina coastal estuarine systems: Newport River, New River and Bogue Sound. Capacity for the average residence time of each system ranged from a low of 0.3 kg of TNT for the Newport River Estuary to a high of 5748 kg for the New River Estuary. These ecosystem capacities did not include any enhanced biodegradation occurring across frontal boundaries but they demonstrated that ambient background level of energetics biodegradation alone may be sufficient to metabolize a substantial release from shoreside. These analyses predicted how much energetics or phenanthrene could theoretically be released into the headwaters of each ecosystem and be attenuated before exiting the estuary in the water column. As other measures of acute toxicity or ecosystem effects were measured that could result from a large scale release were not performed, this is not evidence that there would be no ecosystem effects of a large scale release of any organic carbon, but rather it explains the lack of detection of energetics and PAHs in coastal water and sediment where there were known, recent releases. Taken together, this work supports site conceptual models where PAHs and energetics are rapidly biodegraded by natural microbial assemblages as they migrate from mangrove- or Cypress-dominated systems to adjacent coastal waterways and estuaries. Finally and most significantly, this and our previous SERDP work fully support the emerging understanding that organic matter in coastal and open ocean ecosystems is not recalcitrant based on its chemical structure alone.

DISCLAIMER

The use or mention of commercial products does not constitute endorsement.

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APPENDICES

A) Supporting Data

Taken from ASTMO 2013.

TABLE 1. Sediment sites with reported recontamination.

Site	Response Measure(s)	Recontamination Information	References
Anacostia River, DC	2004 Cap	2006 Urban sources, upstream sources	USEPA 2006
Bloomington, IN (3 creeks)	1987 Sediment Removal	1992 All sources unclear – point source discharge included	ATSDR 1992
Bremerton Naval Complex, WA	2000 Dredge	2000 Losses from CAD placement	SPI 2002, DNO 2002
Convair Lagoon, CA	1998 Cap	2002 Public storm drain discharges	Zeng 2002, Carlisle 2002
Denny Way Site, WA	1990 Cap	1993 CSO point source discharges	Palermo 2002, NRC 2001, Romberg 2005, WDNR 2002
Duwamish Norfolk CSO, WA	1999 Dredge-Cap	2001 CSO point source discharges; unremediated adjacent contaminated sediment	WDE 2003, USEPA 2003
Duwamish River Diagonal, WA	2004 Dredge	2005 Sewage system discharges	SPI 2005
Eagle Harbor Site, WA	1994 Cap	1999 "Surface sources", "offsite sources"	USEPA 1999, Palermo 2002
Ford Outfall/River Raisin, MI	1997 Dredge	2001 Unremediated upstream sediments and/or upland sources; sediments sloughed from adjacent navigational channel	Cieniewski 2003, Bergeron 2000, Cleland 2000, Cleland 2001 Weston 2004
Fox River SMU 56/57, WI	2000 Dredge-cap	2005 1.2-1.5 m of new impacted sediment deposited in five years	AE 2006
Housatonic River, MA	2002 Dredge-Cap	2005 Upstream sediments, CSO and SSO point source discharges	BG 2005
Lauritzen Canal, CA	1996 Dredge-Cap	1998 Undetected point source(s); incomplete remediation near margins of site	USEPA 2001, Weston 2002, USEPA 2004a
Long Beach North Energy Island Borrow Pit (NEIBP), CA	2001 Cap	2004 "Deposition from the surrounding harbor"	USACE 2005
Pier 51 Ferry Terminal, WA	1989 Cap	1990 PAHs due to pile pulling; metals from "new sediment deposition"	HSRC
Pier 53-55, WA	1992 Cap	2002 Prop wash resuspension near edges; PAHs due to pile removal	Romberg 2005
Pier 64-65, WA	1994 Cap	2002 Piling repair work released creosote	Romberg 2005
Puget Sound Naval Shipyard Pier D, WA	1994 Dredge	1998 Suspected resuspension of sediments from outside response area	RETEC 2002
Sitcum Waterway/ Nearshore Tideflats, WA	1993 Dredge	2002 "Continued source input from recent sediment deposition or off-loading activities"	RETEC 2002
St. Clair Shores, MI	2002 Dredge	2003 Sewer pipe discharges	TMD 2006
Thea Foss Waterway, WA	2002 Dredge-Cap	2006 City storm drain discharges	TNT 2006a, TNT 2006b

B) List of Scientific/Technical Publications (ER2124 and SEED)

1. **Osburn, C.L**., Boyd, T.J., **Montgomery, M.T**., Coffin, R.B., Bianchi, T.S., and H.W. Paerl. 2016. Optical proxies for terrestrial dissolved organic matter in estuaries and coastal waters. Frontiers in Marine Science 2:127 DOI: 10.3389/fmars.2015.00127
2. **Osburn, C.L, Montgomery, M.T.,** Boyd, T.J., Bianchi, T.S., Coffin, R.B., Paerl, H.W. 2016. Optical proxies for dissolved organic matter in estuaries and coastal waters. 2016 Ocean Sciences Meeting, New Orleans, LA, 21-26 February.
3. Atar*, J.N., Barnett*, E.L., **Montgomery, M.T.,** Handsel*, L.T., Boyd, T.J., Coffin, R.B**., C.L**. **Osburn.** 2016. Dynamics of fluorescent organic matter compared between three contrasting estuarine environments. 2016 Ocean Sciences Meeting, New Orleans, LA, 21-26 February
4. **Montgomery, M.T.,** Atar*, J.N., Boyd, T.J., Coffin, R.B., **and C.L. Osburn**. 2016. Biodegradation of aromatic contaminants at estuarine frontal boundaries. American Society for Microbiology Microbe 2016 and General Meeting. 16-20 June.
5. **Montgomery, M.T**., Boyd, T.J., Drake, L.A., and **C.L. Osburn**. 2015. TNT degradation by natural microbial assemblages at frontal boundaries between water masses in coastal ecosystems (ER-2124 interim report). US Naval Research Laboratory Letter Report submitted to SERDP (28FEB15).
6. **Montgomery, M.T.,** Boyd, T.J., and **C.L. Osburn**. 2015. PAH and Energetics for natural attenuation rates for current source determination and site closure. Presentation to NAVFAC's Sediment Workgroup, Bremerton, WA, 5 August.
7. Peale, J., Scalzi, M., Fowler, T., **Montgomery, M.T.,** Boyd, T.J., and J.G. Mueller. 2015. Antimethanogenic ISCR reagent for safer, more efficient remedial actions. Presentation at the Third International Symposium on Bioremediation and Sustainable Environmental Technologies. Miami, FL, 18-21 May
8. **Montgomery, M.T.,** Boyd, T.J., Coffin, R.B., Hansel, L.T., Drake, L.A., and **C.L. Osburn**. 2014. TNT degradation by natural microbial assemblages at frontal boundaries between water masses in coastal ecosystems (ER-2124 interim report). US Naval Research Laboratory Technical Memorandum NRL/MR/6110—14-9552
9. Brym*, A., Ziervogel, K., Paerl, H.W., **Montgomery, M.T.,** Handsel*, L.T., and **C.L. Osburn**. 2014. Optical and chemical characterization of base-extracted particulate organic matter in estuaries and coastal waters. Marine Chemistry 162:96-113.
10. Giordano, B.C., **Osburn, C.L,** Lindsey, C., and **M.T. Montgomery,** 2014. Measurement of nitroaromatic explosives by micellar electrokinetic chromatography in waters collected along a salinity gradient of a tropical estuary. US Naval Research Laboratory Technical Memorandum, NRL/MR/6110—14-9504.
11. **Montgomery, M.T.,** Coffin, R.B., Boyd, T.J., and **C.L. Osburn**. 2013. Incorporation and mineralization of TNT and other anthropogenic organics by natural microbial assemblages from a small, tropical estuary. Environmental Pollution 174:257-264.

12. **Montgomery, M.T.,** Coffin, R.B., Boyd T.J., and **C.L. Osburn**. 2013. Degradation of aromatic organic compounds by natural bacterial assemblages at estuarine frontal boundaries. Proceedings of the ASLO 2013 Ocean Sciences Meeting, New Orleans, LA, 17-22 February.
13. Brym*, A.J., Ziervogel, K., Paerl, H.W., **Montgomery, M.T.,** and **C.L. Osburn**. 2013. Characterization of particulate organic matter in three estuaries using parallel factor analysis (PARAFAC). Proceedings of the ASLO 2013 Ocean Sciences Meeting, New Orleans, LA, 17-22 February.
14. **Osburn, C.L.,** Handsel*, L.T., Mikan*, M.P., Paerl, H.W., and **M.T. Montgomery**. 2012. Fluorescence tracking of dissolved and particulate organic matter quality in a river-dominated estuary. Environmental Science and Technology 46, 8628−8636. dx.doi.org/10.1021/es3007723
15. **Montgomery, M.T.,** Coffin, R.B., Boyd, T.J., and C.L. Osburn. 2012. TNT biodegradation by natural microbial assemblages at estuarine frontal boundaries. US Naval Research Laboratory Memorandum Report, NRL/MR/6110—12-9390.
16. **Montgomery, M.T.,** Coffin, R.B., Boyd, T.J., Rose, P.S., Smith, J.P., Sachsenmaier*, L., Mikan*, M., and **C.L. Osburn.** 2011. 2,4,6-Trinitrotoluene (TNT) and aromatic organic matter metabolism by natural bacterial assemblages at estuarine transition zones (ER-2124). Proceedings from the SERDP Partners in Environmental Technology Technical Symposium & Workshop, Washington, DC, November 30-December 2.
17. Luning Prak, D.J., O'Sullivan, D.W., Eisenberg, M.A., **Osburn, C.L., Montgomery, M.T.,** and J.P. Smith. 2011. Photolysis of dinitrotoluene: effects of salinity, nitrate, and humic substances. Proceedings from the SERDP Partners in Environmental Technology Technical Symposium & Workshop, Washington, DC, November 30-December 2.
18. **Montgomery, M.T.,** T.J. Boyd, J.P. Smith, S.E. Walker, and **C.L. Osburn**. 2011. "2,4,6-Trinitrotoluene mineralization and incorporation by natural bacterial assemblages in coastal ecosystems", in, (Eds. M.A. Chappell, C.L. Price, R.D. George) Environmental Chemistry of Explosives and Propellant Compounds in Soils and Marine Systems: Distributed Source Characterization and Remedial Technologies, ACS Symposium Series, Vol. 1069, ACS Publications, Washington, DC, pp. 171-184. DOI:10.1021/bk-2011-1069.ch009.
19. **Montgomery, M.T.**, Coffin, R.B., Boyd, T.J., Smith, J.P., Plummer, R.E., Walker, S.E., and **C.L. Osburn**. 2011. Mineralization rates of 2,4,6-Trinitrotolene and bacterial production amongst natural microbial assemblages in coastal sediments. Environmental Pollution 159:3673-3680. DOI:10.1016/j.envpol.2011.07.018
20. **Montgomery M.T.**, Coffin, R.B., Boyd T.J., Smith J.P., Walker S.E., and **C.L. Osburn**. 2011. 2,4,6-Trinitrotoluene mineralization and bacterial production amongst natural microbial assemblages in coastal sediments. Presentation at 6th International Conference on Remediation of Contaminated Sediments. New Orleans, LA, 7-10 February.

*CLO student

C) Other Supporting Materials

Awards

2014 NRL Chemistry Division Citation for Research Accomplishment and Technical Writing; also Berman Award Nominee 2014:

Montgomery, M.T., Coffin, R.B., Boyd, T.J., and **C.L. Osburn.** 2013. Incorporation and mineralization of TNT and other anthropogenic organics by natural microbial assemblages from a small, tropical estuary. Environmental Pollution 174:257-264.

2012 NRL Chemistry Division Citation for Research Accomplishment and Technical Writing; also Berman Award Nominee 2012:

Montgomery, M.T., Coffin, R.B., Boyd, T.J., Smith, J.P., Plummer, R.E., Walker, S.E., and **C.L. Osburn**. 2011. Mineralization rates of 2,4,6-Trinitrotolene and bacterial production amongst natural microbial assemblages in coastal sediments. Environmental Pollution 159:3673-3680.